THE PRODUCTS OF BIODEGRADATION OF SELECTED CARPET DYES AND DYEING AUXILIARIES

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THE PRODUCTS OF BIODEGRADATION OF SELECTED

CARPET DYES AND DYEING AUXILIARIES

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SUMMARY

Large quantities of effluents containing dyes and other organic compounds are discharged annually by carpet manufacturing operations. Prior to discharge into rivers and streams, these chemicals are generally subjected to biooxidation. The degree of biodegradation and the products produced have not been established for many of these compounds.

The purpose of this research was to determine the percentage removal of selected dyes used in carpet production. The research investigated the percent removal of dyes in a competitive situation where the microorganism could use one of the dyes present or one of the other compounds in the synthetic wastewater as a food source.

This study employed a bench-scale unit as the biooxidation system. The purpose for this system was three-fold. First, the bench-scale apparatus was loaded by a hydraulic system similar to full-scale requirements. Secondly, the preparation of large quantities of influent was not necessary. Finally, uniform organic loading was established with steady-state operating conditions for the biological metabolism of the organic substrates present.

For dye analysis in the synthetic wastewaters two procedures were developed. The first procedure employed column chromatography to recover trace quantities of dyes from large volumes of wastewater. The procedure provided the necessary concentration of the

dyes for chromatographic analysis. The column chromatography technique used also separated the two different classes of dyes studied. The second procedure used was liquid chromatography. The liquid chromatography separated individual dyes within a class and permitted both qualitative and quantitative analysis.

Disperse and acid dyes were investigated in this research. The disperse dyes were recovered from wastewater and analyzed without any serious problems. They were removed in a range from 64 to 97 percent. Problems were encountered in quantitative determinations of acid dyes. Due to these problems actual percentages were not calculated. However, the removal of acid dyes appeared to be lower than that of disperse dyes.

CHAPTER I

INTRODUCTION

A. Definition of the Problem

The carpet industry faces a myriad of stringent rules and regulations with respect to the environment. The guidelines were established in 1972 by the Federal Water Pollution Control Act Amendments. Under these amendments, an industrial permit program initiated by the Army Corps of Engineers was transferred to the Environmental Protection Agency.[1] In accordance with this program, a permit must be obtained certifying that wastewater discharge will comply with prescribed limits.[2] Willful or negligent violations will result in severe fines and possible imprisonment.[3]

Carpet processing wastewater discharge must comply with objectives established by the Environmental Protection Agency specifically for the carpet industry.[4] The EPA has established effluent deadlines for July 1, 1977, the best practical control technology currently available (BPCTCA), and for July 1, 1983, the best available technology economically available (BATEA). Listed in Tables 1 and 2 are the effluent limitations for BPCTCA and BATEA, respectively. These deadlines lean toward the elimination of pollutant discharge into navigable waters by 1985.

In addition to these effluent guidelines, the carpet industry

must pretreat its wastewater under certain circumstances.[5] Pretreatment is necessary when mill effluent contains pollutants incompatible with joint treatment systems. The wastes which require pretreatment before introduction into joint treatment works are those which can cause fires or explosions, obstruct flow, corrode the system, or create excess toxicity. To make mill effluents compatible, specified percentages of any incompatible pollutants must be removed prior to discharge into joint treatment systems.

In the past, carpet dyers have selected dyes for their ability to impart color to the face yarns of carpets economically without consideration of the biodegradability of the dyes. In the future environmental factors must be given greater consideration. Carpet manufacturers must develop methods to effectively treat effluent water from mills and subsequent discharge of the treated water into rivers and streams must not alter "the condition of the water so that it is less suitable for any purpose for which it would be suited in its natural state".[6]

In accordance with Section 307 of PL 92-500 effluent limitations for toxic chemicals will be established as these substances are identified.[7] Potentially pollutant dyes in carpet mill effluents wastewater will be identified. Limitations will then be established based upon the toxicity of each dye. By-products from the degradation of these dyes will also be limited based upon their toxicity.

TABLE 1

Guidelines For
Best Practical Control Technology Currently Available

EFFLUENT CHARACTERISTIC:	Effluent Limitation
BOD's:	Maximum for one day: 8.6 kg/1000 kg product. Maximum average of daily values for any period of 30 consecutive days: 4.3 kg/1000 kg of product.
COD:	Maximum for 1 day: 60 kg/1000 kg of product. Maximum average of daily values for any period of 30 consecutive days: 30 kg/1000 kg of product.
TSS:	Maximum for any one day: 8.6 kg/1000 kg of product. Maximum average of daily values for any period of 30 consecutive days: 4.3 kg/1000 kg of product.
pH:	Within range of 6.0 to 9.0.
FECAL COLIFORM:	MPN shall not exceed 400 counts per 100 ml.

TABLE 2

Guidelines for
Best Available Technology Economically Achievable

EFFLUENT CHARACTERISTIC:	Effluent Limitation
BOD's:	Maximum for any 1 day: 5.8 kg/1000 kg of product. Maximum average of daily values for any period of 30 consecutive days: 2.9 kg/1000 kg of product.
COD:	Maximum for any 1 day: 16 kg/1000 kg of product. Maximum average of daily values for any period of 30 consecutive days: 8.0 kg/1000 kg of product.
TSS:	Maximum for any 1 day: 5.8 kg/1000 kg of product. Maximum average of daily values for any period of 30 consecutive days: 2.9 kg/1000 kg of product.
pH:	Within range of 6.0 to 9.0.
FECAL COLIFORM:	MPN shall not exceed 400 counts/100 ml.

B. Brief History Leading to the Problem

The production of carpets has increased tremendously in volume, since its introduction as a popular floor covering. Carpet sales volume is predicted to increase 10% annually through 1980.[8] Concurrent with the increase in processing, there will be an increase in the volume of wastewater discharged by processing mills.

In recent years the concern for the detrimental effects on water quality due to carpet processing has been intensified by increased carpet production. In 1974, carpet industries used 22 billion gallons of water.[9] Compared to the 1973 estimate of 1.2×10^{13} gallons of water used by all industries,[10] the carpet industry accounted for approximately 0.2% of the total water used. Therefore, concern for water pollution created by carpet production originates from the variety of chemicals used and their concentrations rather than the amount of water used in processing. Approximately 50% of all carpet production in the United States is located in Northwest Georgia[9] which further concentrates the pollutants discharged by carpet mills in a small regional area.

Until 1972 water pollution legislation had been lenient. The stringent guidelines established in 1972 seemed so extreme due to the short time span to remedy the problem that the task seems unrealistic to some,[11] and irrational to others.[12] This amendment may reflect "the premise that if not enough is demanded in the beginning,

then little will be gained in the end".[12] Throdahl of Monsanto states "outside the offices of the EPA itself, industry may be the most effective supporter of the act". But he also believes "the rigid guidelines need to be more flexible in order to incorporate pertinent information accumulated through research and development".[13] The carpet industry has the obligation to the public to protect the environment. To do this, Barnhart states, "we have to go back to the basics and find out what is in the wastewater. We must identify the incompatible chemicals, the true refractories and separate them for recycling, perhaps, and treat the residue to a high level".[14] The biodegradability of dyes is an important part of the information needed for effective decision-making in regard to carpet processing wastes.

Little data exist on the biodegradabilities of dyes in waste treatment systems. Several earlier studies have shown that selected dyes biodegrade when subjected to activated sludge treatments.[15,16] These studies did not investigate the competitive biodegradability of dyes in wastewater with other processing chemicals. Batch type bench scale systems were used in the studies to avoid working with large quantities of water.[9]

Since carpet production is indigenous to Georgia, and the waste treatment system of Dalton carries approximately 25% of all carpet dyeing waste in the U.S., this system was chosen as a typical system treating wastewater consisting mainly of carpet mill effluents. Approximately 30 million gallons of wastewater are treated each day by the Dalton waste treatment facility. Around 80% of this water is

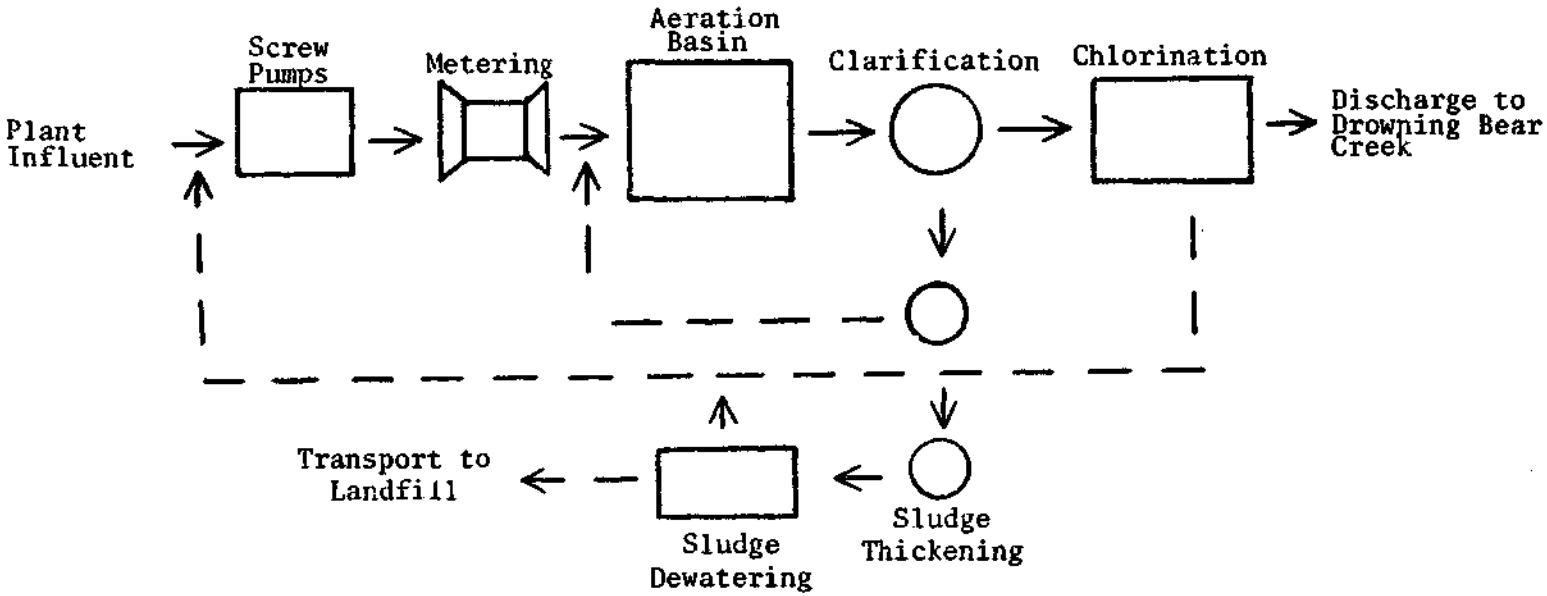


Figure 1. Flow Diagram of the Dalton, Georgia Waste Treatment Facility.

from carpet processing.[17] A continually fed bench scale system was developed to serve as a model for the Dalton Waste Treatment Plant (Figure 1). Another reason for choosing the Dalton facility is that it releases its effluent into the Coosa River Basin. This is of vital importance, because numerous towns in Georgia and Alabama receive their water supply from this river basin.[18]

C. Objective of the Research

The intent of this research was to determine the percent removal of selected disperse and acid dyes used in carpet processing. This research investigated the removal of dyes in a competitive system where the microorganisms could degrade the dyes present or one of the other chemicals present in the synthetic wastewater. A bench scale system with uniform organic loading was established with steady state operating conditions for the biological metabolism of the organic substrates present.

CHAPTER II

LITERATURE REVIEW

The synthetic organic chemicals used in carpet processing are of special interest for several reasons. One is the variation in structure of the chemical compounds. Another area of interest is the degradation processes of these chemical compounds in nature.[19] This interest stems from the fact that these materials are not naturally occurring and their degradation pathways in natural environments are difficult to predict. Dyes are a major concern in this group because of their ability to impart color to water at very small concentrations. For instance, each of the disperse and acid dyes used in this study could readily be seen visually at a concentration of 1 PPM or less.

One area of research reported in the literature is the effect of dyes on aerobic systems. Hunter showed that 17 of 46 dyes studied caused a definite inhibition of activity in aerobic systems.[20] Disperse Blue 7 exhibited adverse effects on the effectiveness of activated sludge in removing pollutants from water. The inhibition of biological activity was believed to be dependent on the chemical structure of the dye molecule and the concentration of the dye.

The toxicity of dyes is another important aspect of their presence in wastewater. Wilkinson has shown that dyes can interfere with normal metabolic pathways.[21] In tests performed by Lamb and

Little, some dyes were found to be toxic to the fathead minnow.[22] Of all the dyes tested, the triphenylmethane dyes, Basic Violet 1 and the Basic Green 4 were found to be the most toxic. Several disperse dyes, Disperse Yellow 42, Yellow 3, Yellow 54, Blue 7, and Red 60 were found to be toxic at concentrations greater than 180 mg/l. Acid dyes were found to be toxic at levels as low as 3.2 mg/l. Similar to the acid dyes, basic dyes exhibited toxic properties at low concentrations. From these results it appears that the toxicity of the different classes of dyes may be influenced by their ionic character.

Large amounts of data concerning the biological treatment of wastewater containing dyes have been accumulated over the past several years. Most of these data describes the effect on color of gross operational parameter changes in systems used for wastewater treatment. These data describes the results due to the retention time, primary, secondary, and tertiary treatments, and combination of various treatments. Several of these systems will be discussed below.

Other data files have been collected from the results of standard testing procedures. These tests are analytical methods to determine the acceptability of water for its intended use after treatment.[23] Table 3 includes some of the more common tests with average values from the influent and effluent of the Dalton waste treatment facility.[17] This treatment facility handles wastewater with approximately 85% of its influent originating from carpet mills. The values in Table 3 are useful measures indicating removal of the original pollutants in the wastewater. However, these tests do not determine

TABLE 3

Data of the Dalton, Georgia Waste Treatment Plant

TEST	INFLUENT	EFFLUENT
BOD_5 (mg/l)	212	8.2
COD (mg/l)	546	111
Color (APHA units)	520	167
pH	6.8	6.8
Phenols (mg/l)	.15	.04
Suspended Solids (mg/l)	95	21.2
Dissolved Soldis (mg/l)	917.9	390.9
Chromium	0	0

which pollutants are being removed. Therefore, they are of little value where information is needed on specific pollutants.[24]

Activated sludge, trickling filter, and stabilization ponds are the three main systems of biological wastewater treatment. Activated sludge removes many organic compounds from wastewater by biological flocculation.[24] It is a common system used in treating carpet mill effluents.[25] This is one of the least expensive ways to treat wastewaters, but it has an instability problem caused by cyclic flow into the waste treatment facility. This problem is also common in trickling filters and stabilization ponds. Trickling filtration is similar to the activated sludge process, but the wastewater is sprayed onto a bed of crushed rock, or other media, coated with biological films. The trickling filter suffers from problems due to cold weather, odor, organic overloading, and filter flies. Stabilization ponds treat wastewater effectively, but they have severe odor problems, and require extensive amounts of land.[26]

Many physical and chemical methods to remove dyes from wastewater are being investigated. Research in this area encompasses several techniques. Several of these systems are discussed below.

Hyperfiltration has the potential of removing dyes from wastewater. One investigation has shown that 87% of water used in processing could be recovered for reuse with the removal of 99% of the color.[27] Cost is one of the major problems in using reverse osmosis as a system for treating wastewater. Other problems are encountered due to membrane deterioration, and the pumping and pressure equipment.[27]

Significant reduction of color, COD, total organic carbon, and suspended solids were reported after ozone treatment of the effluent from the secondary clarifier of an activated sludge system. Ninety percent of this wastewater was from tufted carpet dyehouses.[28] In other studies, disperse dyes were removed from dyeing process wastewater by first mixing the effluent with a strong acid or alkali, and then decolorizing the water by ozone treatment.[29] The oxidation of azo dyes in alkaline solution has also been investigated.[30] These studies showed that azo dyes could be degraded by oxidation with ozone in alkaline solution.

Adsorption of dyes on activated carbon and polymeric materials has been shown to be an effective method of removing dyes from wastewater.[31,32] Adsorption on polymeric materials is an efficient tertiary system removing 90-98% of the color in wastewater.[32] Although carbon is efficient, difficulties arise in regenerating the activated carbon for continued use. Studies using combinations of ozone treatment with activated carbon have reported synergistic effects in the removal of dyes from wastewater.[33]

Other techniques of tertiary treatments investigated include ion exchange, electrodialysis, and distillation. Ion exchange is selective in its function, removing 80-92% of the nitrogen and phosphorous present. This system costs about 20¢ per 1,000 gallons of water treated. Electrodialysis was low in efficiency (10-40%) for the removal of dissolved solids (at high cost).[31] Economic considerations have hindered the development of distillation procedures for the

removal of dissolved solids from wastewater. Work in this area has been toward the development of methods to transfer the heat of the steam to a circulating oil bath which can be used for other carpet processes, such as drying.[34]

Solvent processing and cycling spent dyebaths are two methods currently being investigated to reduce the pollutant discharge from carpet mills. Recycling spent dyebaths reduces pollution by reusing the same water several times with chemicals being replaced when necessary.[35] Solvent systems regenerate the solvent after processing, thus reducing pollutant discharge. At the present time, all of these advanced treatment systems and alternate dyeing systems are still in the experimental or early development stage. Biological treatment is expected to continue to be the major technique for treatment of textile wastewater.

The biodegradation of selected anthraquinone disperse dyes has been reported in earlier studies.[16] Disperse Blue 7 was metabolized 60% biologically, but no evidence of the ring system being degraded was found. Another study reported the biodegradation of azo disperse dyes by activated sludge.[15] The cleavage of the azo linkages of selected disperse dyes was reported in this study. The biodegradation of these azo dyes yielded colorless aromatic amine metabolites which appeared to be toxic to the microorganism if the food supply was inadequate. These studies showed that anthraquinone and azo disperse dyes could be biodegraded. Two characteristic environmental factors were not investigated in these studies. One was continuous organic

loading. According to Busch,[36,37] these feeding systems were not conducive to the high level of microbial activity reached in steady-state conditions.[36,37] Another factor, that was characteristic of the environment, was competitive biodegradation. In each of the previous studies mentioned, the feedings of the batch system consisted of only one dye from the variety of chemicals used in carpet processing, thus forcing the sludge to metabolize the dye as a food source. In this work the activated sludge received a continuous flow of synthetic wastewater containing a large variety of chemical compounds used in carpet production. This should be more indicative of actual waste treatment systems giving the sludge an option of using dyes, or other chemical compounds, or both as food sources.

Once the dyes have been subjected to activated sludge treatment, a method of recovery and analysis of each dye quantity present in the effluent of the system is necessary. The method of recovery and analysis often consist of several steps. The normal procedure is:

1) The recovery of the dyes from the wastewater
2) Concentration of the dyes to improve analytical results
3) The separation of the various classes of dyes
4) The analysis of each dye in a class.

In the literature, the recovery of dyes from aqueous solution has been accomplished by the adsorption of dyes on macroreticular resins (Amberlite XAD-2).[38] This system also permits concentration of the dyes, a necessary procedure for chromatographic analysis using

liquid chromatography. Selected acid and disperse dyes were reported to have recoveries of 80-100% using the XAD-2 resin in column chromatography. The reported procedure also separated acid and disperse dyes. Liquid chromatography appears to be a useful technique for separating and analyzing the disperse and acid dyes investigated in this research.[39-41]

CHAPTER III

EXPERIMENTAL PROCEDURE AND INSTRUMENTATION

A. The Biooxidation System

1. Synthetic Sewerage

The synthetic wastewater subjected to activated sludge treatment in this research was designed in such a way that competitive biodegradation could be investigated. The chemical compounds used were chosen from a report listing the types of compounds and their quantities discharged by the carpet industry.[9] The concentration of dissolved solids for synthetic sewerage was determined by the following equation:

$$2x\left[\frac{\text{Total Lbs. of Chemicals Discharged by the Carpet Industry/Year}}{\text{365.25 Days/Year}} \times 0.25 + 21.5 \text{ mg/Day}\right] \times \left[4.536 \times 10^5 \frac{\text{Milligrams}}{\text{Lb.}} \div 3.785 \frac{\text{Liters}}{\text{Gal.}}\right] \quad (1)$$

The coefficient 2 increased the concentration of dissolved solids to a value more representative of typical wastewaters. The fraction of the total chemical discharge by all carpet industry that was discharged in Dalton is represented by the figure 0.25. The dissolved solids concentration calculated was 515.4 mg/l. Suspended solids were not used in order to avoid the settling problems which would be incurred.

In Table 4, the chemicals and their concentrations in the original influent prepared for this study are listed. The original influent was in error. It was found that the latex compounds were removed by primary treatment system in industry rather than biological system.[42]

The list of chemicals used in the synthetic waste was revised omitting the latex compounds. Bacto Peptone was substituted in the place of the latex compounds. Originally the Bacto Peptone had been used to simulate the domestic waste in the synthetic sewerage. The substitution of the peptone was to keep the dissolved solids at a constant level.

2. Static Aeration System

A static aeration system was used to acclimate the sludge from the Dalton waste treatment plant to laboratory conditions. The wastewater was the same as that described previously, except the concentration was increased by a factor of 2. This was done to increase the amount of sludge collected for seeding the continuous aeration system. Two liters of synthetic wastewater were seeded with one liter of sludge collected from the clarifier of the waste treatment facility in Dalton. The system was then mixed and aerated by mechanical means.

The basin was continually aerated until the chemical oxygen demand (COD) was reduced to a constant value. The constant COD value was used as the criterion to indicate the end of removal of oxidizable material. The sludge developed in this batch system was used to seed a second static aeration system. The sludge developed in the second

TABLE 4

Chemicals in the Synthetic Waste and Their Concentrations (mg/l)

Fiber Finish	
Sandoz Sanolube	32.6
Fiber Production	
Caprolactum	32.1
Other Processing Chemicals	
Mineral Oil	19.9
pH Control	
Monosodium phosphate	24.9
Tri- and tetra sodium phosphate	35.1
Acetic acid	15.5
Sodium carbonate	3.8
Diammonium hydrogen phosphate	0.5
Ammonium acetate	12.8
Sodium Hydroxide	2.1
Ammonium Sulfate	6.7
Carrier	
Chemkar 603	8.6
Auxiliaries	
Defoam 721-T	38.4
Migrassist NYL	15.6
Igepal CA630	20.3
Igepal CO630	20.3
Plexene D	1.6
Chemcoloft	0.3
Alkanol CN	6.6
2-ethylhexanol	24.6
Benzyl alcohol	40.9
Celca gum D70D	37.5
Celca gum D47D	37.5
Nofome J	0.1

TABLE 4 (cont.)

Diluents	
Morasperse N22	3.2
Reax 85A	3.2
Tamol SN	3.2
NaCl	8.1
Sugar (Table)	8.1
Latex Compounds	
Trimene Base	9.4
Zinc mercaptobenzothiazole	9.4
Zinc diethyldithiocarbonate	25.3
Dyes (Commercial)	
Disperse Yellow 23	1.2
Disperse Yellow 3	0.7
Disperse Yellow 54	0.6
Disperse Red 60	0.6
Disperse Red 55	0.2
Disperse Blue 7	0.5
Disperse Blue 120	0.2
Acid Yellow 19	0.4
Acid Yellow 151	0.3
Acid Yellow 135	0.3
Acid Red 151	0.3
Acid Red 337	0.3
Acid Orange 128	0.2
Other Compounds	
Bacto Peptone	1.4

system was used to seed the continuous biooxidation system.

3. Continuous Biooxidation System

A continuous activated sludge system was chosen to study the removal of selected carpet dyes from wastewater. Activated sludge is a common method used to treat wastewater prior to discharge into waterways. The apparatus used (Figure 2) as the biooxidation system in this research was originally designed by Busch.[36] The one actually used in this study was produced by the Horizon Ecology Company. It provides a system for uniform organic loading. This should improve the correlation between bench scale experimental data and the data collected from pilot and full scale operations.

The uniform organic loading was provided by the continuous flow inherent to this system. A Masterflow pump head was used to pump the influent into the 6-liter Vitax outer cone (Figure 2). The effluent was removed by a Masterflow pump larger than the influent pump to prevent overflow. Circulation and aeration were provided by three special fritted glass diffusers. The air flow (10 cubic feet per hour) carried mixed liquor up between the 2-liter inner cone and the 6-liter outer cone. Air bubbles were released at the surface and the liquid recirculated by a downflow in the inner cone. A 1.5-inch outer diameter cylindrical clarifying tube suspended in the center of the inner cone provided a method to separate the effluent from the sludge.

The continuous aeration system was seeded by decanting the entire static system into its aeration basin. The remaining volume of

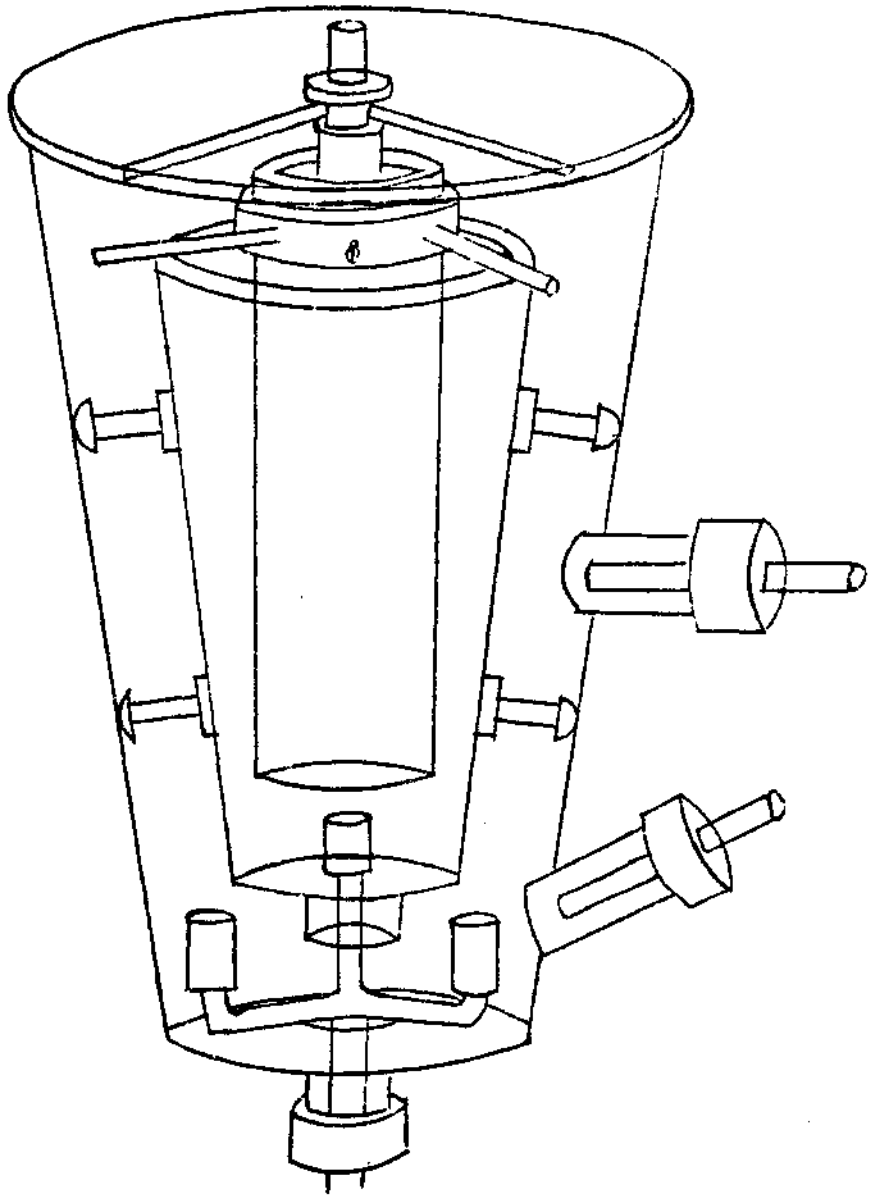


Figure 2. Biooxidation Apparatus.

the aeration basin was filled with the revised synthetic waste described by equation 1.

The retention time of the waste liquor in the aeration basin was 17.82 hours. This time was calculated from design data provided by the Georgia Environmental Protection Department.[43] This retention time was calculated using data for 0% return sludge. This allowed longer periods for the waste liquor in the aeration basin. Although sludge was not recycled after removal from the aeration basin, it was recycled internally while in the basin.

Chemical oxygen demand[44] was used as the criterion for establishing equilibrium in the aeration basin. In this study, a 30.0 ml sample of wastewater was refluxed with known amounts of potassium dichromate, and sulfuric acid thus destroying most types of organic matter present. The excess dichromate was then titrated with ferrous ammonium sulfate. The amount of dichromate consumed, provided a measure of the oxygen equivalent of the portion of organic matter in the sample that is susceptible to oxidation. The activated sludge was in a steady state condition when the COD of the effluent reached a constant level.

The dissolved oxygen in the influent and effluent were recorded using a Model 57 Dissolved Oxygen Meter (Yellow Springs Instrument Company). Corning Model 12 pH Meter was used to record the pH of the influent and effluent wastewaters. A record of the temperature in the aeration basin was also maintained. The sludge concentration was determined using an Imhoff cone.

B. Dye Recovery and Analysis

Following treatment by activated sludge the dyes had to be removed from the wastewater for analysis. This was accomplished by column chromatography which also provided the necessary increase in concentration of the dyes for subsequent analysis. After recovery and concentration, the dyes were analyzed by liquid chromatography.

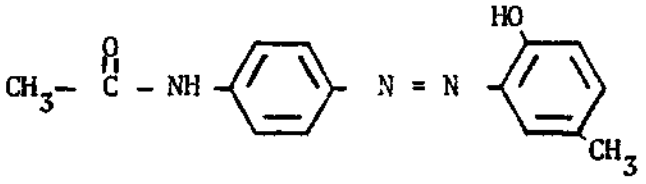
1. Column Chromatography

In this research the concentration of dyes in the wastewater was expected to be low, 1 mg/l or less. Column chromatography provided a method to recover and concentrate the acid and disperse dyes examined in this study. Typical structures for the dyes investigated are given in Table 5.[45] Structures of all the dyes used in the study have not been reported. Basic structures are given in Table 5 for these dyes. Column chromatography was also investigated as a method to separate the acid and disperse dyes.

a. Column Preparation

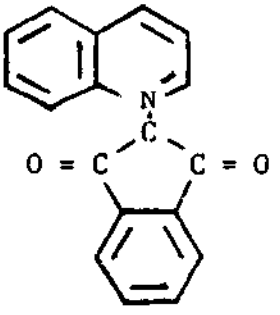
Dyes were removed from wastewater by adsorption on a column of macroreticular resin. The columns were prepared in 9mm x 500mm glass columns (Lab-Crest) with detachable teflon stopcocks or teflon needle valves. A glass wood plug was placed in the bottom of the column to macroreticular resin. Approximately 30 ml of Amberlite XAD-2 resin

Table 5. Structures of Acid and Disperse Dyes Investigated

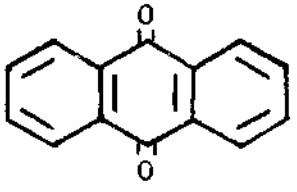


Disperse Yellow 3

Disperse Yellow 23

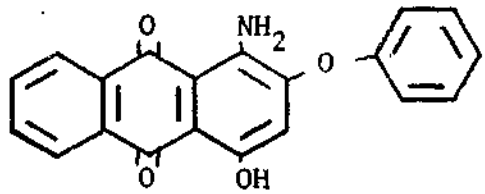


Disperse Yellow 54

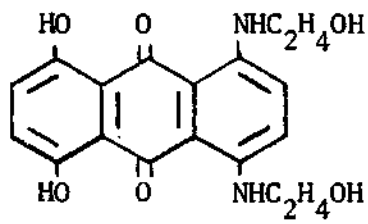


Anthraquinone (Representative of
Disperse Red 60 and Blue 120)

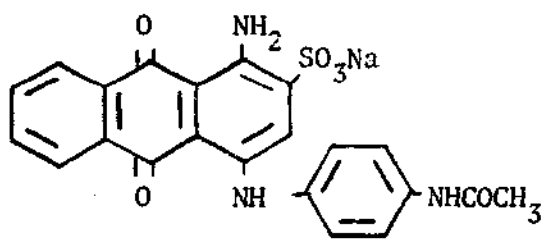
Table 5 (cont.)



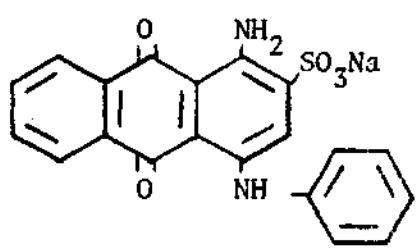
Disperse Red 60



Disperse Blue 7

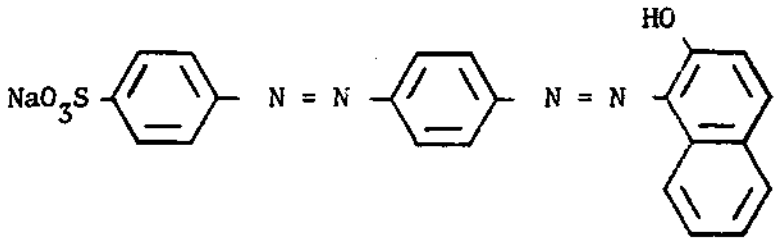


Acid Blue 40

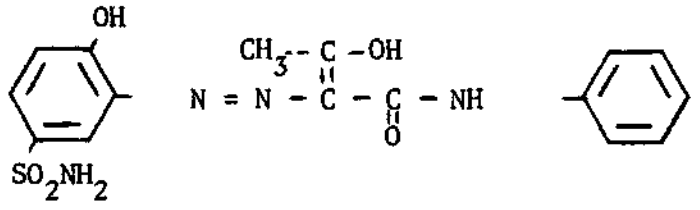


Acid Blue 25

Table 5 (cont.)

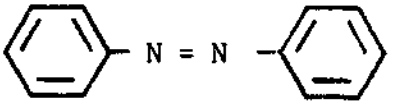


Acid Red 151



Acid Yellow 151

Diazo Compound (Representative of Acid Orange 128)



Monoazo Compound (Representative of Acid Red 337, Acid Yellow 135, and Acid Yellow 19)

(Rohm and Haas), 20-50 mesh, was slurried in approximately 60 ml of methanol. A methanol layer was maintained above the resin as it was poured quickly into the column (5 minutes). The column was then rinsed with 40 ml of benzene at approximately 2 bed volumes per hour. The benzene wash was followed by an upflow of 40 ml of methanol at 8 bed volumes per hour. This was done for several reaons. First, it served as a washing process. Second, it promotes the removal of air from the column, and third, it "classifies" the column. Finally it was used as an intermediate between benzene and the next wash solvent. Following the methanol rinse, the column was washed with a pyridine: tetrahydrofuran:1% ammonium hydroxide (40:40:20)(v/v/v) solution at a flowrate of 4 bed volumes per hour. The column was giving a final rinse with 200 ml of distilled water at 4 bed volumes per hour. A glass wool plug was placed on top of the resin to prevent it from shifting when the column was inverted. The column was stored under distilled water until used.

The layer bed volume, 30 ml, was used to preference to smaller bed-volumes (15 ml). Significant improvement was seen in the recovery of Disperse Yellow 54 using the larger amount of resin (Table 6). Disperse Yellow 3 and Disperse Yellow 23 were recovered within acceptable limits using either the small or large resin volume. Disperse Yellow 54 was not recovered within the necessary range using the 15 ml bed volume, but recovery with a 30 ml bed volume was acceptable.

A criteria of 70% dye recovery was used in this study as an

TABLE 6

Effect of Bed-Volume On Dye Recovery

	Percent Recovery Bed Volume	
	15 ml	30 ml
Disperse Yellow 23	76	77.5
Disperse Yellow 3	82	97.7
Disperse Yellow 54	55	76.5

acceptable limit. This limit was established to yield an overall recovery of approximately 50% for the two analytical procedures. It was felt that 50% was a minimum acceptable limit for dye detection.

b. Dye Removal from Wastewater

The dyes were removed from wastewater samples by passing a solution of wastewater and N,N-dimethylformamide over the resin. The dimethylformamide was added to increase the solubility of disperse dyes so they were absorbed on the resin. If dye was present in the wastewater, colored bands appeared in the upper regions of the column.

Silicon tubing (1 inch) was used to attach a 1000 ml Kelly infusion jar to the top of the column (Figure 3). Wastewater (900 ml) and dimethylformamide (100 ml) were placed in the infusion jar, and allowed to flow through the resin at 4 bed volumes per hour. After all the wastewater had passed through the column, the reservoir and column were washed with 50 ml of a 90 water-10 dimethylformamide mixture, thus rinsing the Kelly infusion jar and insuring that all the wastewater sample had contacted the column.

c. Dye Removal from the Column

After the dyes were recovered from wastewater samples, a procedure for extraction and collection of the dyes was required. In this study, the solvent system used both recovered and separated the acid and disperse dyes.

After the wastewater sample was passed through the column, the

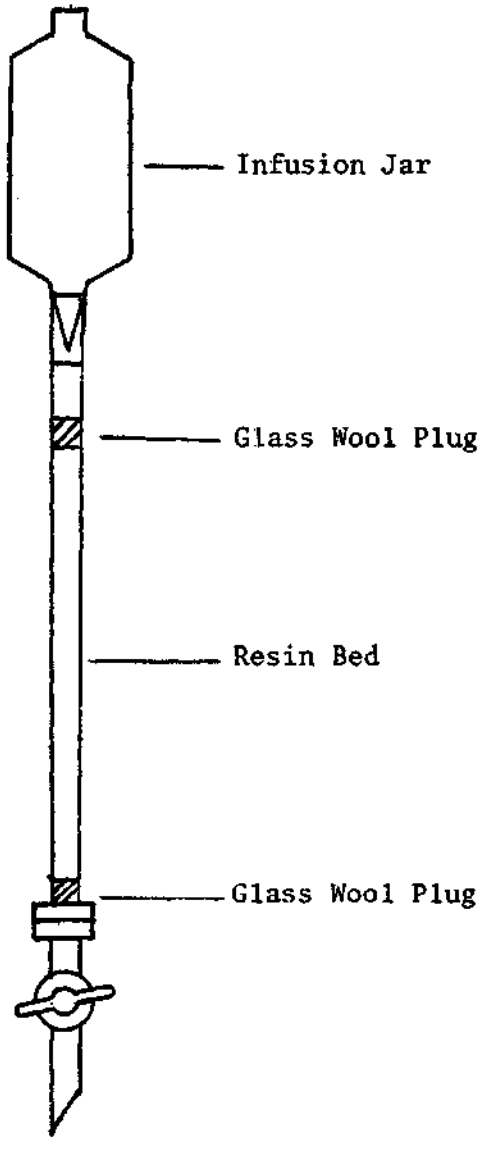


Figure 3. Column Chromatography for Wastewater Samples.

reservoir and stopcocks were removed and the column inverted for extraction processes. The column was inverted, since the dye had been sorbed in the upper regions of the resin. A 9mm x 500mm extension column was attached to the top to serve as a solvent reservoir.

The column after inversion was first eluted with 40 ml of benzene at approximately 2 bed volumes per hour. The disperse dyes were removed by benzene and the acid dyes remained on the column. The benzene containing the disperse dyes was collected in a 50 ml round bottom flask with a 24/40 ground glass joint. The benzene was removed on a Buchi Rotovap R under aspirator vacuum at temperatures up to 100°C. The disperse dye was taken up in a 1/99 (v/v) dimethylformamide/benzene solvent mixture and diluted to 25 ml in a volumetric flask. The solution was now ready for separation and quantitation by liquid chromatography.

The acid dyes were difficult to remove from the column. Initially, methanol was used to remove the acid dyes. Although methanol could recover several dyes, it could not be used to recover Acid Yellow 19, Acid Blue 40, Acid Red 151 and Acid Orange 128 (see Table 7). Several solvents and solvent systems were used to remove acid dyes from the resin. They included methanol, dimethylformamide, chloroform, 1% ammonium hydroxide, 1% formic acid, tetrahydrofuran, and pyridine. Some results of the study are shown in Table 7. The quaternary ammonium solution using ammonium hydroxide/pyridine/tetrahydrofuran was found to be the most successful in extracting acid dyes from the resin.

TABLE 7

Solvents and Solvent Systems for Acid Dye Removal From Columns

SOLVENTS OR SOLVENT SYSTEM	DYE	% RECOVERY
1) Methanol (40 ml)	Acid Yellow 19	0
2) 60 $CHCl_3$:40 Methanol (40 ml)	Acid Red 151	7
3) a) Dimethylformaide (80 ml)		
b) Methanol (40 ml)	Acid Red 151	75*
	Acid Yellow 19	30
4) a) Dimethylformamide (80 ml)		
b) Methanol (40 ml)		
c) 1 NH_3:99 H_2O (40 ml)	Acid Yellow 19	44
5) a) 1 NH_3:99 H_2O (40 ml)		
b) Methanol (40 ml)	Acid Red 151	42
6) a) HCOOH (40 ml)		
b) Tetrahydrofuran (40 ml)	Acid Red 151	31
7) a) HCOOH (40 ml)		
b) Tetrahydrofuran (40 ml)	Acid Yellow 19	32
8) a) 1 NH_3:99 H_2O (40 ml)		
b) Pyridine (40 ml)	Acid Yellow 19	41
9) a) Methanol (40 ml)		
b) 40 THF:40 Pyridine:20(1 NH_3:99 H_2O) (40 ml)	Acid Yellow 19	70*

*Acceptable recovery - 70% or greater.

The acid dyes were removed after the disperse dyes by first eluting with methanol (40 ml) followed by 40 ml of the 40/40/20 (v/v/v) pyridine/tetrahydrofuran/(1% ammonium hydroxide) mixture, both at flowrates of 4 bed-volumes per hour. The methanol and solvent mixture extracts were combined in a 250 ml round bottom flask and rotovapped as described for the benzene extracts. The acid dyes were taken up in a 1-part dimethylformamide to 99-part methanol solvent mixture, and made up to 25 ml in a volumetric flask. These acid dye solutions were then ready for analysis in the liquid chromatograph. Thus, the column chromatography step served to separate the acid and disperse dyes, and provided a 36-fold increase (900 to 25 ml) in dye concentrations.

d. Efficiency of Column Chromatography

After the recovery procedure was developed, percent recoveries for each of the dyes were determined. The recovery for each dye was important to insure that reliable results were obtained in later analysis.

Determination of the recovery efficiency required preparation of pure dye samples. Since commercial dyes contain additional compounds to improve their dyeing ability, they must be purified for analytical work. Separate purification procedures were required for acid and disperse dyes.

An extraction procedure was used to purify disperse dyes. Five grams of a disperse dye were placed in a cellulosic extraction thimble (Whatman, 34mm x 93mm) and placed in a soxlet extractor. A water

jacketed condenser and a 300 ml boiling flask containing 200 ml of benzene was attached to the extractor. The benzene was refluxed until the filtrate appeared colorless. The benzene containing the pure disperse dye was collected in the boiling flask. The flask was placed on a Buchi Rotovap R and benzene removed under aspirator vacuum at temperatures up to 100°C. The pure dye was recovered by scraping the flask with a spatula. The dyes were stored in test tubes with teflon coated screw caps until needed.

Acid dyes were purified based on their solubility in methanol. Two grams of a commercial acid dye were dissolved in 100 ml of methanol. This solution was filtered through cellulosic filter paper. The filtrate was collected in a 250 ml round bottom flask equipped with a 24/40 ground glass joint. The methanol was removed on a Buchi Rotovap R under aspirator vacuum at temperatures up to 100°C. The recovered dye was dissolved in 100 ml of methanol, filtered and again recovered. This procedure was repeated until no residue appeared on the filter paper. The acid dyes recovered in the final step were stored in glass tubes with teflon screw caps until needed.

The next step in determining the column efficiency for each dye was the development of working curves of absorbance versus concentration. A Beckman DB-G Recording Spectrophotometer with 1 cm quartz cells was used to obtain the spectrophotometric data. The disperse dyes were dissolved in 99/1 (v/v) benzene/dimethylformamide and the acid dyes in 99/1 (v/v) methanol/dimethylformamide. The dimethylformamide was used to increase the solubility of the dyes in their respective solvents.

Spectra giving absorbance versus wavelength were recorded with the DB-G (see Appendix A). From these plots the wavelength of maximum absorption was determined for each dye. Spectra of the disperse dyes and acid blue dyes were recorded at 10 parts per million (ppm). Spectra of the remaining acid dyes were recorded at 5 ppm due to their higher tinctorial strengths. In Table 8 the wavelength of maximum absorbance are listed for each dye in this research.

Graphs of concentration versus absorption were prepared for each dye (Appendix B). These graphs were obtained at the maximum absorbance wavelength for each dye. From the Beer-Lambert Law, A = abc, where a is the extinction coefficient of each dye, and b is a constant thickness of the sample, the concentration is proportional to the absorbance. Therefore, the concentration of an unknown solution of each dye may be determined by the following equation.

$$\text{Concentration of Unknown Dye Solution} = \frac{\text{Absorbance of Dye Solution}}{\text{Slope of Absorbance vs. Concentration for that Dye}} \qquad (2)$$

The final steps in determining the column efficiency included extraction of the dyes from water solutions on XAD-2 resin removal of the dyes from the resin, and determining the amount of dye recovered. Two-hundred milliliters of a 1 mg/l solution containing one of the pure dyes in 90/10 (v/v) distilled water/dimethylformamide were passed through the column at 4 bed-volumes per hour. This was followed by a 50 ml wash of 10/90 (v/v) dimethylformamide/water as described in

TABLE 8

Disperse Dyes	Wavelength (Maximum Absorption)
Blue 7	627 nanometers
Red 55	510
Blue 120	618
Yellow 3	402
Yellow 54	447
Yellow 23	385
Red 60	515
Acid Dyes	
Yellow 135	400 nm
Red 337	509
Orange 128	414
Yellow 19	418
Red 151	510
Yellow 151	440
Blue 25	625
Blue 40	623

Wavelength of Maximum Absorption for Each Dye.

previous sections. The column was inverted and the extraction procedure performed as previously described:

1) 40 ml of benzene at 2 bed volumes per hour
2) a) 40 ml of methanol at 4 bed volumes per hour
   b) 40/40/20 (v/v/v) tetrahydrofuran/pyridine/(1% ammonium hydroxide) at 4 bed volumes per hour

The eluted dye was dried using the Buchi Rotovap, and redissolved in 20 ml of the respective solvents for analysis on the DB-G. The concentration of the final solution was determined, and the percent recovery calculated by Equation 3.

$$\text{Percent Recovery} = \frac{[\text{Dye}]_{\text{final}}}{10 \times [\text{Dye}]_{\text{initial}}} \times 100 \qquad (3)$$

This procedure was repeated for each acid and disperse dye.

e. Wastewater Samples

Effluent wastewater samples were taken from the bench scale bio-oxidation system to determine the quantities of disperse and acid dyes remaining in the wastewater after treatment. These samples were taken over a twenty-day period with the samples collected on alternate days.

The samples were obtained by collecting 900 ml of wastewater as it flowed up the clarifier tube. The wastewater was prepared for column chromatography by mixing 900 ml of the effluent with 100 ml of dimethylformamide. An influent sample was prepared by the same method.

The dyes were recovered from the wastewater, removed from the column, and prepared for injection into the liquid chromatograph by procedures described earlier.

Samples were taken from the Dalton waste treatment facility and the stream into which it discharges. Samples from the Dalton system were taken from the influent at the lift pump, and the effluent of the clarifier. These samples were also concentrated by resin adsorption, recovered and prepared for injection into the liquid chromatograph.

2. Liquid Chromatography

High pressure liquid chromatography was chosen for the quantitative separation and quantitation of dyes recovered from wastewater samples. In this method, dyes to be separated were dissolved in a solvent and pumped onto a small column containing absorbant particles with a high surface area. The differing partition of the dyes between the stationary phase (column) and the moving solvent phases resulted in the separation of the dyes as they moved down the column. The dyes were detected as they were eluted from the column by a visible wavelength spectrophotometric detection system. The system was selected due to its ability to separate organic molecules with only slight differences in chemical structure.

The instrument used in this research was the Micromeritics Model 7000 Liquid Chromatography (Figure 4). This instrument could be operated in either constant pressure or constant flowrate modes of operation. The instrument contained two pumping systems, so that two solvents

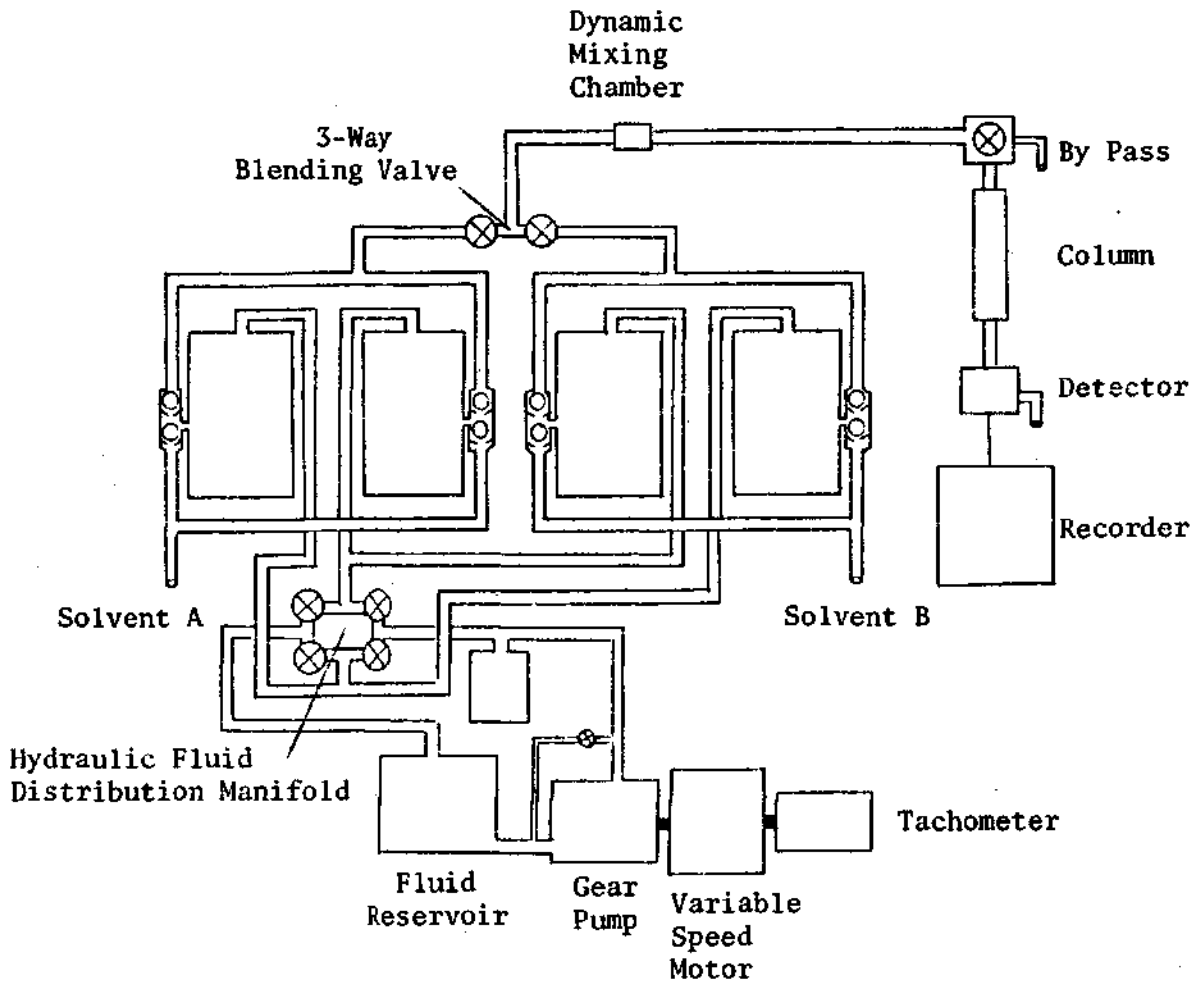


Figure 4. Model 7000 Series Chromatograph Operation Schematic.

could be mixed internally by adjusting the ratio from 0%A-100%B to 100%A-0%B. The pumps were controlled by solvent programs so that a solvent gradient of any selected slope could be generated. The mobile solvents were high purity methanol, tetrahydrofuran, and cyclohexane (Burdick and Jackson Laboratories, Inc.) and distilled water.

The eluted dyes were detected by a Tracor Model 970 Variable Wavelength Detector using a quartz-iodine lamp. Some experiments were also conducted using a Varian Vari-chrom with a tungsten light source. The data were recorded on a Perkin-Elmer Model 56 strip chart recorder.

a. Calibration

The liquid chromatograph was calibrated by the same procedure for each acid or disperse dye. The instrument was adjusted to specific settings for each dye. These adjustments will be discussed in following sections. The column was preconditioned with the proper solvent or solvent mixture. Following this, three injections were made with dye concentrations of 5, 10 and 20 mg/l. The peak from each injection was traced on white paper. The tracings were cut from the paper, and the area under the curve weighed. The dye concentration versus absorbance (weight) was graphed completing the calibration procedure. The retention times were measured in the standard sample injections for identification purposes.

b. Disperse Dyes in Liquid Chromatography

Disperse dyes were separated and quantitated by liquid chromato-

graphy using a constant flowrate mode of operation. A Partisil-10 PAC column by Whatman was used to separate these dyes. This column contains 10 μm silica particles with cyanopendant groups, and is designed to separate organic compounds of intermediate to high polarity. Cyclohexane and tetrahydrofuran (weak/strong) were used as the solvent mixture for separating the disperse dyes. The following wavelengths were used for dye detection: (1) 420 nm for yellow dyes, (2) 520 nm for red dyes, and (3) 620 nm for blue dyes. More detailed instrument specifications for each dye is given below.

1. Disperse Red Dyes. Initially a deuterium lamp was used as a light source in the Micromeritics Detector for study of the red dyes. They were injected into the liquid chromatograph using several weak/strong solvent ratios to locate the dyes. The detection of the red dyes was adequate for analytical work at concentrations on the order of 100 mg/l as can be seen in Figure 5. The sensitivity was not high enough for the predicted concentrations of the red dyes in wastewater samples. The Varian Varichrom detector was then examined. The sensitivity with this detector was improved enough to find the optimum solvent concentrations for each red dye. Disperse Red 55 was eluted with an 80/20 (v/v) tetrahydrofuran/cyclohexane solvent mixture and Red 60 with a 35/65 (v/v) tetrahydrofuran/cyclohexane mixture. Finally, the Tracor 970 was chosen for the study, because it was more sensitive to the red dyes at 520 nm.

A solvent gradient mode of operation was not used with the red

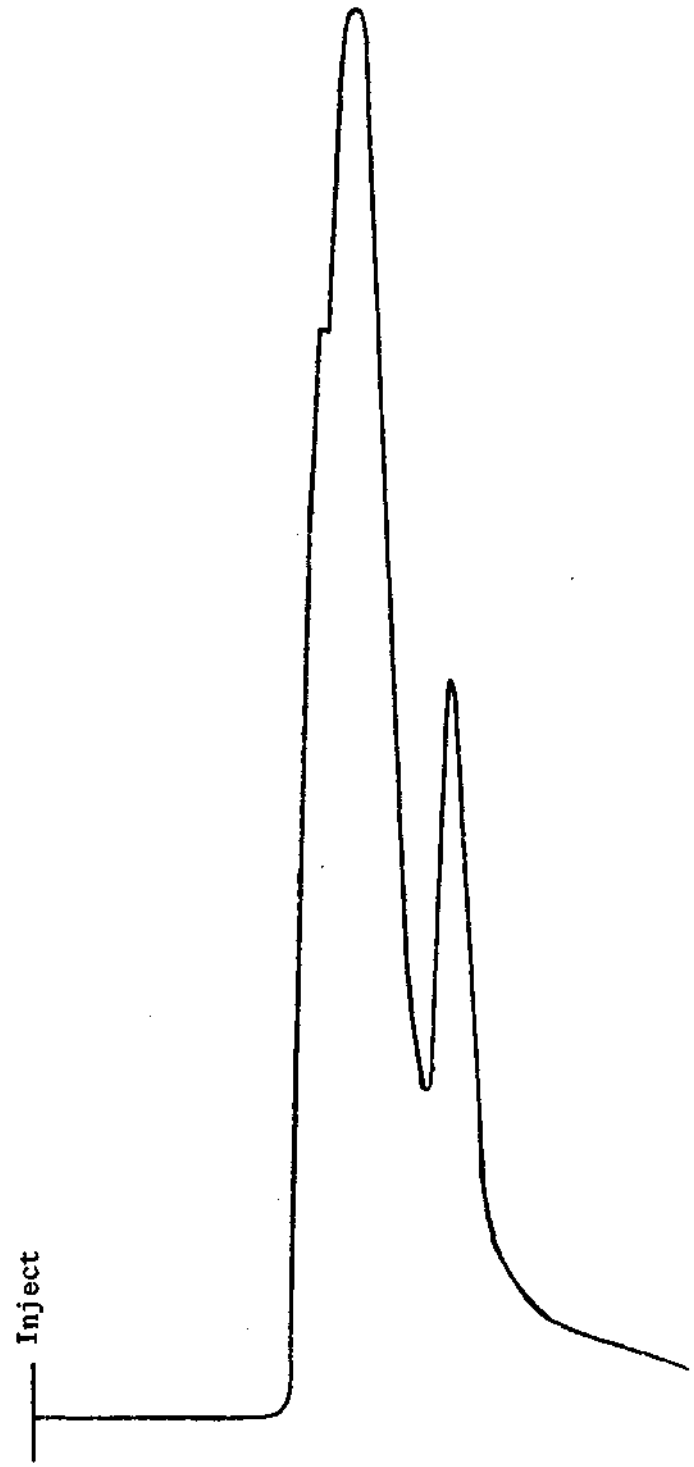


Figure 5. Disperse Red 60 - Liquid Chromatogram.

dyes. This was due to the decrease in intensity of the peaks with the gradient system. The probable reasons for this was that the dyes were mixtures of several compounds which were separated with the long retention times of gradient systems.

Initially, 100 mg/l solutions of the red dyes in 1/99 dimethylformamide/benzene were injected into the liquid chromatograph to determine the optimum solvent concentrations and instrument settings for each dye. A flowrate of 1 ml/minute and an injection size of eight microliters were selected. The detector sensitivity was set at 0.04 units full scale for Red 60 and 0.02 for Red 55 with the data being recorded at 10 millimeters per minute chart speed.

Two separate runs were required to analyze the red dyes on the liquid chromatograph. At 35 tetrahydrofuran/65 cyclohexane Red 60 was eluted one to two minutes after the solvent front. Red 55 was retained on the column at these conditions. Using an 80 tetrahydrofuran/20 cyclohexane mixture, Red 60 was eluted with the solvent and Red 55 appeared one to two minutes later.

Thus the following procedure was used for separation and quantitation of the red dyes. The instrument was adjusted to the settings mentioned previously, depending on the dye being examined. The column was then equilibrated fifteen minutes with the initial solvent mixture. The standard samples were injected to calibrate the instrument. Immediately following calibration wastewater samples were injected. These samples included the effluent and influent samples from the bench scale bio-oxidation system and samples of wastewater from the Dalton area. The

absorbance was recorded for each injection by cutting out the absorbance versus time curves and weighing the area.

The efficiency of column-liquid chromatography system was determined for each dye in the following manner. A bench scale influent sample was subjected to column chromatography to extract the dyes. The dyes were recovered, put in solution, and injected into the liquid chromatograph. This procedure used the techniques outlined earlier. The percent efficiencies were determined by Equation 4.

$$\text{Percent Efficiency} = \frac{\text{Dye Detected by Liquid Chromatography}}{\text{Dye Actually Present}} \times 100 \quad (4)$$

The actual amount of dye present was determined using Equation 5.

$$\text{Commercial Dye in Influent} \times \% \text{ Purity} \times 36 = \text{Dye Actually Present} \quad (5)$$

The percent purities are listed in Table 9 for disperse and acid dyes. The disperse dyes were assumed to be 22% pure when the actual figure was not known. The acid dyes according to manufacturers are more pure, so an average value of 50% was assumed.[9]

The percentage removal was determined for the red dyes by the following technique. The dye concentration in the bench scale effluent was determined. Equation 6 was then used to calculate the percent removal.

$$\text{Percent Removal} = \frac{\text{Dye in Influent} - \text{Dye in Effluent}}{\text{Dye in Influent}} \times 100 \quad (6)$$

This procedure was repeated for each of the ten samples from the bench

TABLE 9

Percent Purity of Dyes[9]

Disperse Dye	Percent Purity
Yellow 3	22
Yellow 23	19
Yellow 54	25
Red 60	16
Red 55	22
Blue 7	22
Blue 120	22
Acid Dye	
Yellow 19	50
Yellow 135	50
Yellow 151	50
Red 151	50
Red 337	50
Orange 128	50
Blue 25	50
Blue 40	50

scale system for each red dye.

2. Disperse Blue Dyes. The disperse blue dyes were the most difficult to determine quantitatively in this research. The dyes investigated were Disperse Blue 7 and Blue 120. The difficulty in analysis was believed to stem from two primary reasons. One was the phototube. Above 600 nm the low response of the phototube could not be used to calibrate the instrument for blue dyes. The second reason believed to contribute to the difficulties encountered was that each blue dye consisted of several components. This was observed in the chromatographs of each blue dye (Figures 6 and 7). Disperse Blue 7 consisted of five major components (Figure 6), and Blue 120 is shown to consist of at least five components also in Figure 7.

Using the Tracor detector Blue 7 was detected and calibrated by the liquid chromatograph. A 100 mg/l solution of Blue 7 in 1/99 (v/v) dimethylformamide/benzene was used to optimize the liquid chromatograph for detecting this dye. The operating conditions chosen were:

1) Solvent System - 100% tetrahydrofuran
2) Flowrate - 1 ml/minute
3) Injection Size - 25 microliters
4) Detector Sensitivity - 0.01
5) Wavelength - 620 nm

The retention times for the two major peaks of Blue 7 were used for identification.

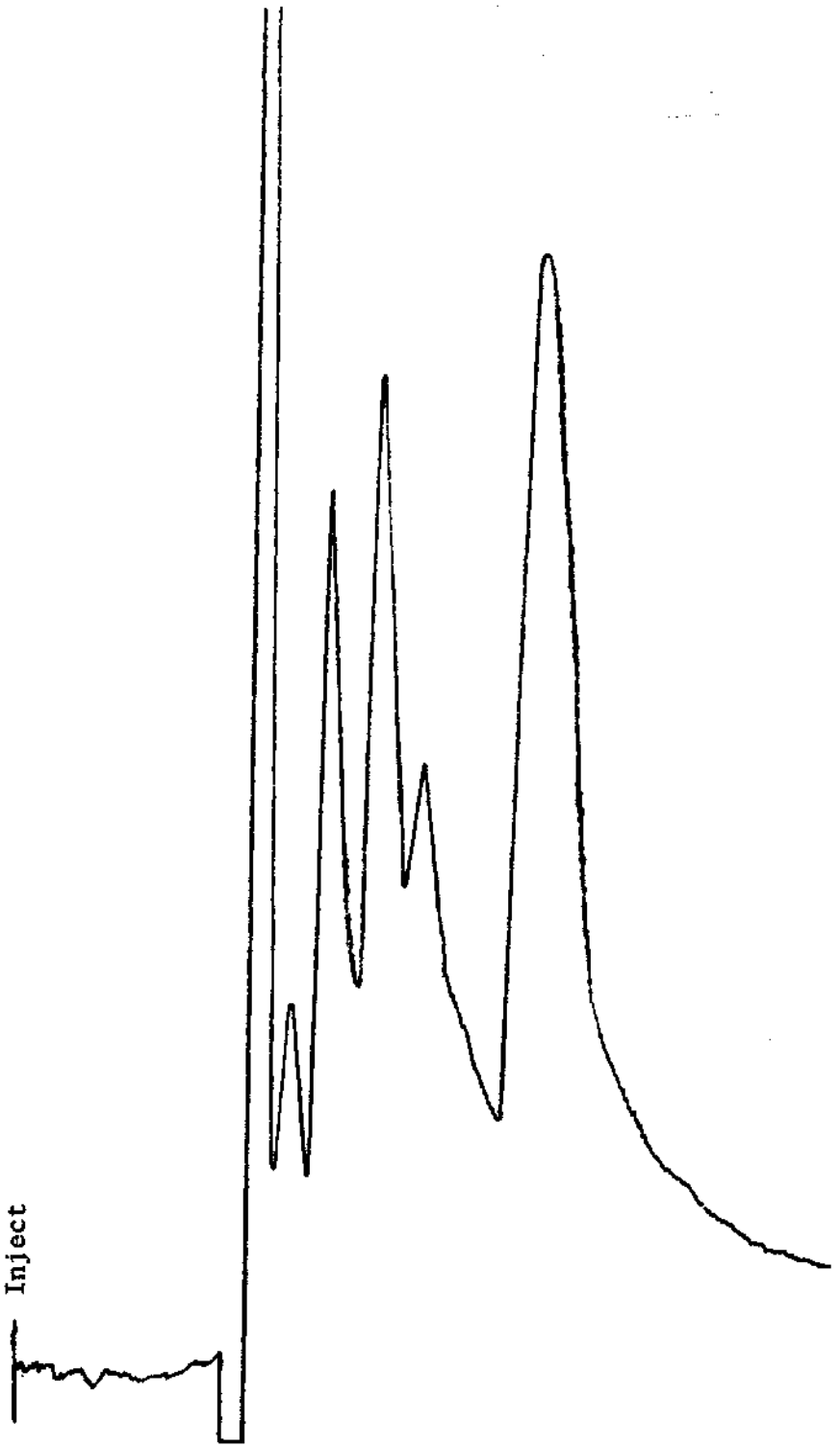


Figure 6. Liquid Chromatogram of Disperse Blue 7.

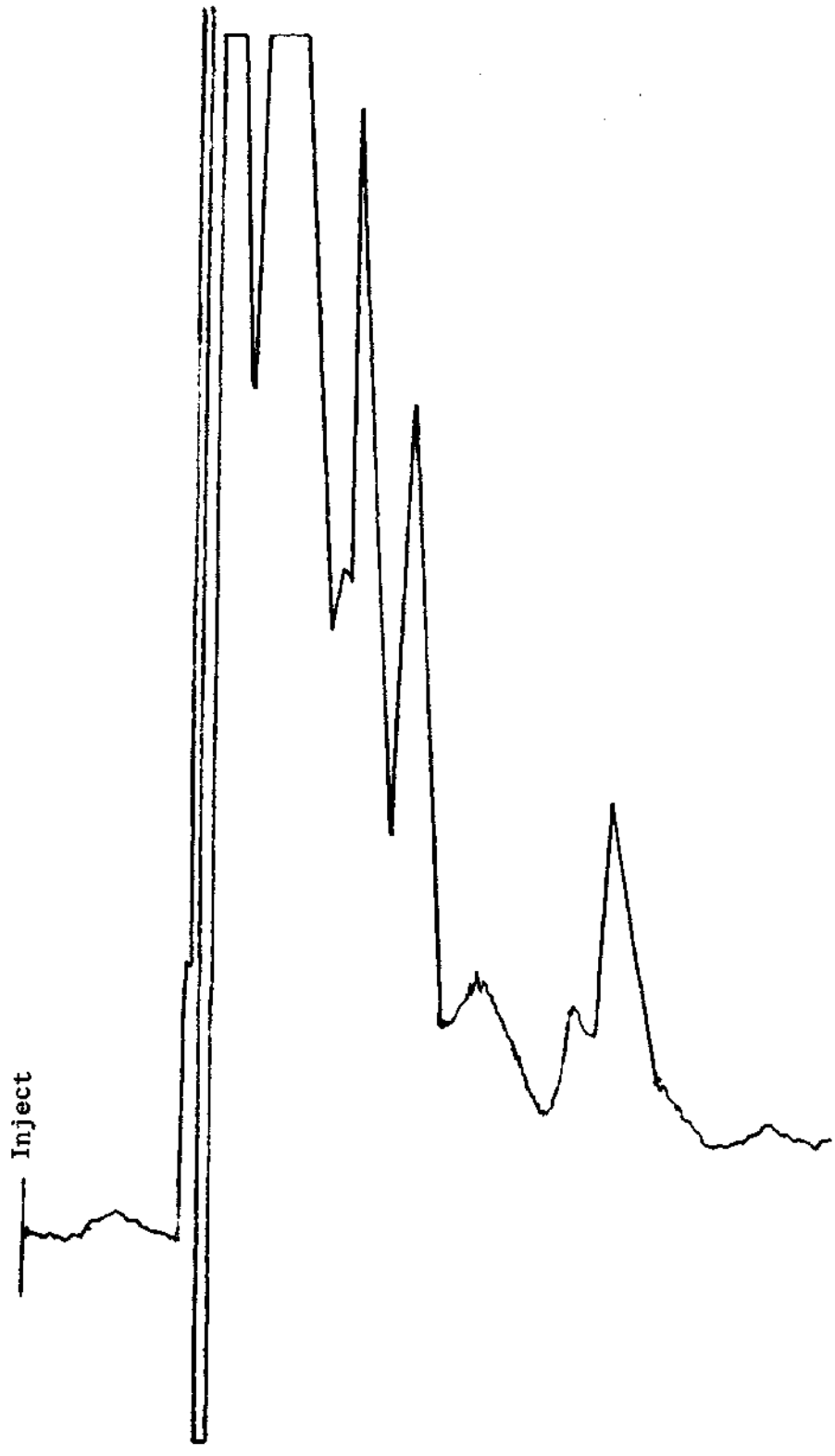


Figure 7. Liquid Chromatogram of Disperse Blue 120.

Solutions of Blue 7 at concentrations of 5, 10, and 20 mg/l were used to calibrate the detector. Graphs of absorbance (determined by weight) versus concentration were plotted for each of the two major peaks of Blue 7 shown in Figure 8. Wastewater samples were injected immediately following calibration. The absorbances of each of the two major components were recorded. The samples were the same as those described in the section for red dyes. The techniques were also the ones used in the study of the red dyes.

The efficiency of the column-liquid chromatography system for Blue 7 analysis, and the percent removal were both calculated using techniques described for the red dyes. Each peak of Blue 7 was treated separately. Working curves of absorbance (weight) versus concentration were developed for each peak. The efficiency and percent removal of each peak were determined for all the samples as described for the disperse red dyes. These were determined by making the proper substitutions into Equations 4 and 6.

3. Disperse Yellow Dyes. The three disperse yellow dyes investigated in this research were Yellow 3, Yellow 23, and Yellow 54. All three detectors employed in this study were used for the quantitation of the yellow dyes. It was found that the yellow dyes could be detected and calibrated by the liquid chromatograph using any of the detectors available. The Varian detector with a tungsten lamp was used because it was available at the time the disperse yellow dyes were being investigated.

Initially it was determined that the three yellow dyes were par-

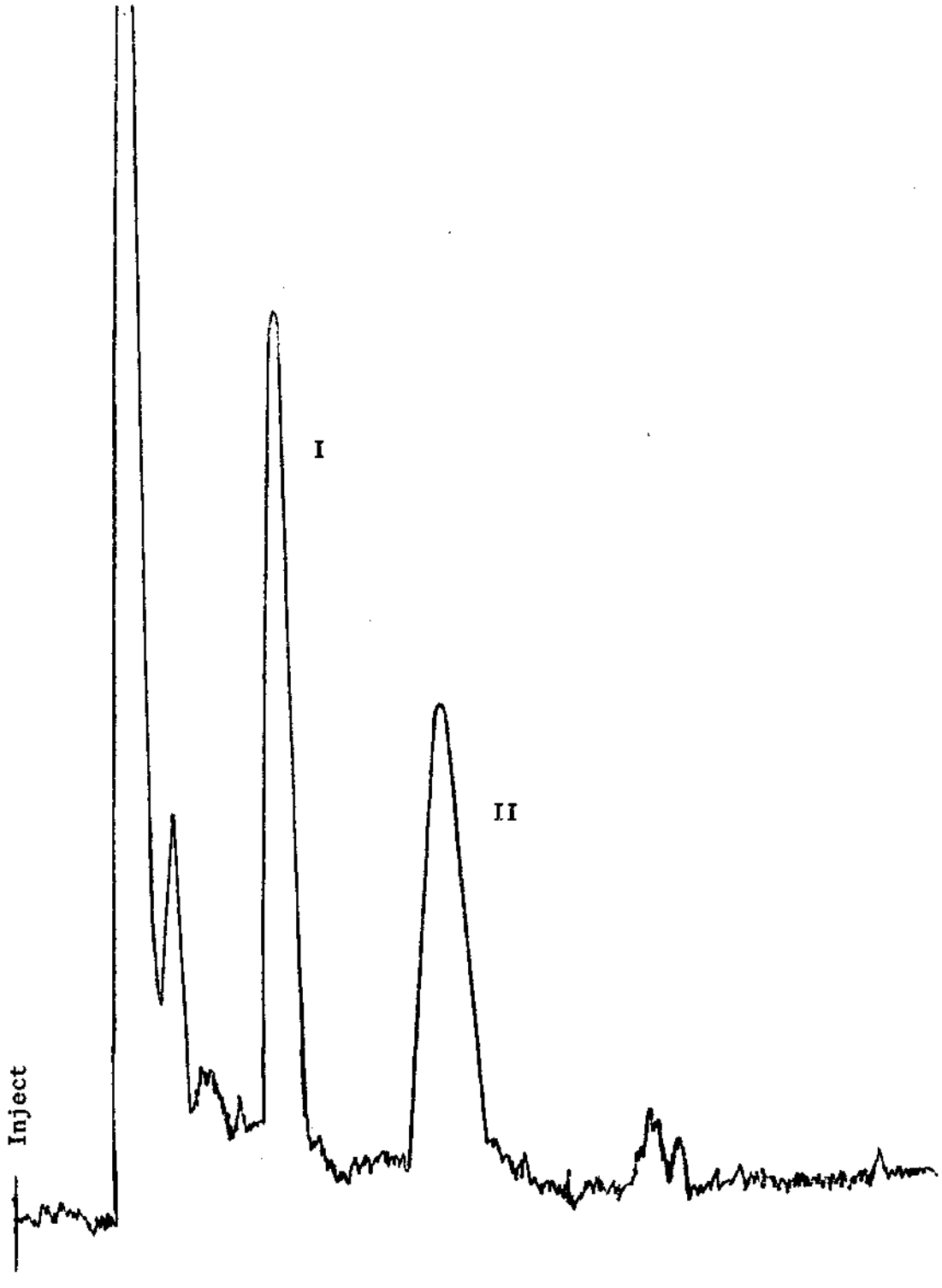


Figure 8. Chromatogram of Disperse Blue 7 from Liquid Chromatography.

titioned by the column in liquid chromatography using different weak/strong solvent mixtures. A study to determine if the three yellow dyes could be separated and eluted using one solvent mixture in an isocratic mode then followed. This system failed. The dyes more strongly bound to the column were not detected with weaker solvent mixtures. When stronger solvents were used, the dyes less tightly bound to the column were not resolved from the solvent front. Finally, linear gradient systems were investigated to examine the possibility of analyzing the three yellow dyes by one injection into the liquid chromatograph. This proved to be a feasible technique. After several injections at various solvent mixtures, gradient ranges and times the optimum operating conditions were determined. They are as follows:

1) Solvent systems - 25 tetrahydrofuran/75 cyclohexane to 100 tetrahydrofuran in 15 minutes (linear gradient)
2) Flowrate - 1 ml/minute
3) Injection size - 9 microliters
4) Sensitivity - 0.5
5) Wavelength - 420 nm

The order of appearance of the dye peaks was the same for isocratic and gradient systems. Yellow 54 was eluted from the column followed by Yellow 23 and then Yellow 3 (Figure 9). The retention times of these dyes were measured as a means of identification.

The instrument was calibrated by injecting three solutions containing each of the dyes at concentrations of 5, 10, and 20 mg/l in a

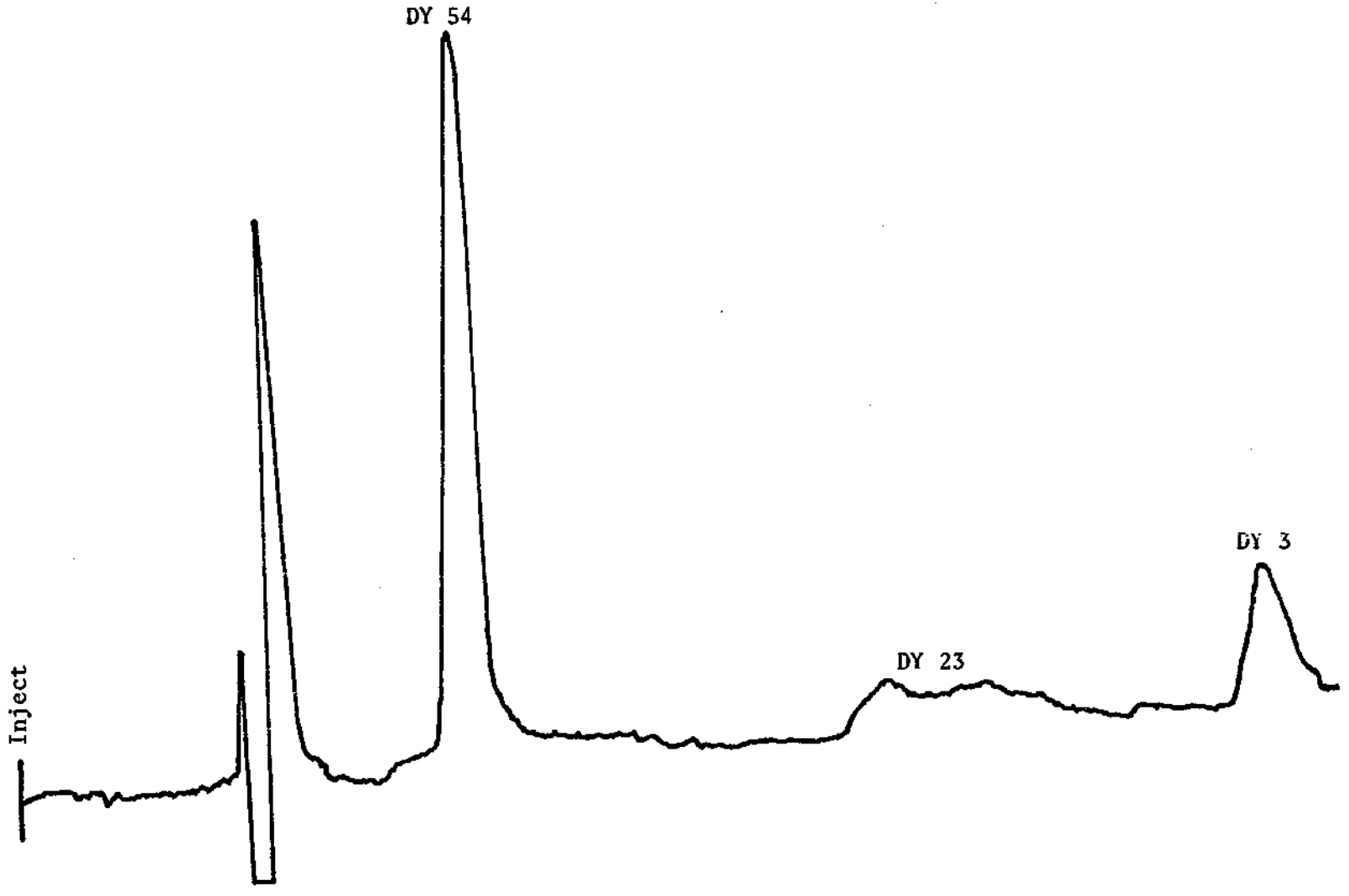


Figure 9. Liquid Chromatogram of Disperse Yellow Dye.

1/99 (v/v) dimethylformamide/benzene solution. Plots of absorbance versus concentration were made for each of the disperse yellow dyes.

The wastewater samples were injected immediately after calibration. These samples were from the influent and effluent of the bench scale biooxidation system, and the Dalton area. The absorbances of each sample solution were recorded for each of the peaks occurring at the retention time of one of the three yellow disperse dyes.

The efficiencies of the column-liquid chromatography procedure used in the analysis of the three yellow disperse dyes were determined using Equations 4 and 5. The proper substitutions were made into these equations to determine the overall efficiency of the system for the recovery and analysis of each dye.

The percent removal of each dye was calculated from the collected data. Equation 6 was used making necessary substitutions for each of the yellow dyes. These percentages were calculated for each of the dyes for the samples taken from the bench scale activated sludge effluent.

c. Acid Dyes in Liquid Chromatography

Acid dyes were investigated by liquid chromatography using a technique known as paired-ion chromatography. The column used for separation was a Partisil PXS 10/25 ODS-2 column by Whatman. The packing material was silica particles bonded with C_{18} hydrocarbon groups. The acid dyes were eluted with methanol-water mixtures containing tetrabutylammonium phosphate. The same wavelengths were used for the detectors in acid dye analysis as used in disperse dye analysis.

1. Preparation of Solvents. Special precautions were taken in the preparation of the solvents used as the mobile phase in acid dye analysis. The first precaution was to filter the solutions because the tetrabutylammonium phosphate would form a precipitate after a period of time. The other precaution was the deaeration of the solvents prior to use. This was necessary due to the tendency of methanol-water mixtures to release air bubbles after being pressurized.

To prepare these solutions PIC Reagent A, tetrabutylammonium phosphate, (Waters Associates, Inc.) was added to water and to methanol. These solutions were then filtered through a millipore filter. Filter type FH (.5μm) was used for the methanol solution and filter type HA (.45μm) was used for the water solution. These filtrations were made using aspirator vacuum. The solvents were then mixed in the proper ratios for analytical work. The mixtures were poured into a round bottom flask (2000 ml). The flask was attached to a water jacketed condenser and the mixture heated to boiling, and allowed to reflux for five minutes. The heat was then removed and the solution allowed to cool. Once boiling stopped, the solution was carefully decanted into a clean bottle with a teflon screwcap. The cap was placed on the bottle and the mixture allowed to cool to 20°C. The solvent mixture was then ready for use.

2. Acid Dye Analysis by Liquid Chromatography. Most acid dyes contain at least one sulfonate substituent group, therefore paired-ion chromatography was a useful technique. In this system, positively charged ammonium ions become associated with the negatively charged

anions of the dyes. This decreases the interaction of the dyes with the column packing material, and as the strong solvent ratio is increased the dyes become more soluble in the mobile solvent phase.

The acid dyes were investigated by liquid chromatography using the same instrument settings for each dye with the exception of the absorbance range and wavelength of light. The wavelength of light was chosen depending on the color of dye being studied. The wavelengths used were as follows:

1) Acid Blue Dyes - 615 nm
2) Acid Red Dyes - 520 nm
3) Acid Yellow Dyes - 420 nm

The absorbance ranges for the dyes were adjusted to give maximum peak areas with the peaks remaining on scale.

The solvents were premixed to reduce the differences between the weak and strong solvents. Thus the weak solvent consisted of 60/40 (v/v) methanol/water with PIC Reagent A, and the strong solvent was a mixture of 85/15 (v/v) methanol/water with Reagent A. For analysis, the column was equilibrated with the weak solvent for fifteen minutes. The sample was then injected. As the sample was injected, the solvent gradient was initiated. The gradient changed the solvents from 100% weak to 100% strong in a period of ten minutes. The other instrument settings were the flowrate (1 ml/minute) and injection size (8 microliters).

The liquid chromatograph was calibrated by injecting standard samples and plotting area (weight) versus concentration for standard dye solutions. The samples were then injected as previously described,

and all data recorded. The percentage removal was calculated using Equation 6, and the system efficiency determined by Equations 4 and 5 using the technique described previously for the disperse dye analysis.

CHAPTER IV

RESULTS AND DISCUSSION

A. The Biooxidation System

The static system was used to acclimate the sludge to laboratory conditions. The batch system was monitored until the chemical oxygen demand reached a constant level. This sludge was then transferred to another static aeration basin, and the system monitored until the COD reached a constant value. In figure 10 a continuous time versus COD plot is shown for both batch systems. At the time the COD had reached a constant value, the sludge concentration was 95 ml/l with a turbid brown appearance.

The continuous aeration system was seeded with the sludge developed in the static systems. When the uniform organic loading was begun, foaming was a serious problem. After several weeks of continuous operation, foaming ceased to be a problem. This was an indication of the sludge being acclimated tc the continuous loading of organics in this system. Concurrent with the decrease of foaming was a change in the color and appearance of the oxidation basin. Initially the basin had a brown color and appeared transparent. As the sludge became acclimated it changed colors from brown to yellow and then it became a turbid brown.

Initially the average effluent COD was approximately 400 mg/l. After three weeks of continuous operation there was a decline in the level of the effluent COD to an average value of 230 mg/l. This was also an

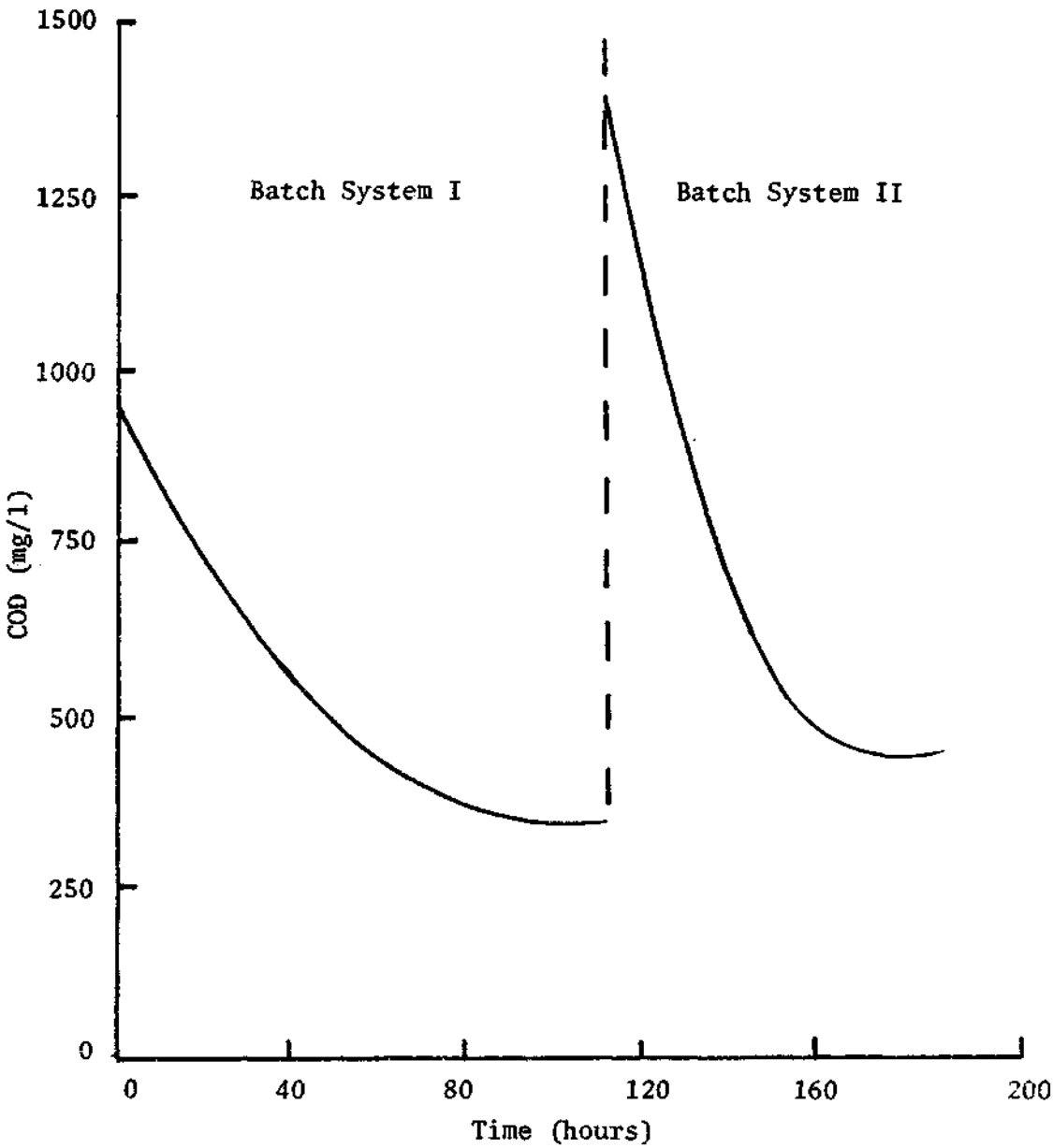


Figure 10. Chemical Oxygen Demand of the Batch Aeration System.

indication of the sludge being acclimated to the continuous biooxidation system.

Sludge was not intentionally wasted from the system, due to its insignificant amount. Although the sludge was not removed directly, there was a loss of approximately 10 ml of sludge per liter of effluent liquor as determined using the Imhoff cone.

Data for the characterization of the bench scale activated system is shown in Table 10. The results are from the 10 days that wastewater samples were taken for degradation studies. As seen in Table 10 the sludge maintained an average operating equilibrium COD value of 234 mg/1. The pH remained nearly constant at near neutral. Dissolved oxygen concentrations showed a significant increase in the effluent compared to influent concentrations. The percent COD removal for the bench scale system was 62. This was less than the average value of 80% for the Dalton treatment facility, which was derived from COD values of 546 mg/1 in the influent and 111 mg/1 in the effluent. The pH of the Dalton system was constant for the influent and effluent at 6.8, which correlated with the value of the bench scale system of 6.9 for influent and effluent. The concentration of dissolved solids in the influent of the Dalton treatment plant was 517.9 mg/1.

B. Column Chromatography

To study the percent removal of dyes in competitive situations, a method to recover and concentrate the dyes was needed. Column chromatography was investigated as a technique for this purpose.

Column chromatography proved to be an acceptable technique for

TABLE 10

Characteristic Data of the Bench Scale Biooxiation System

Sample	Effluent COD (mg/l)	Dissolved Influent (mg/l)	Oxygen Effluent (mg/l)	pH		T (°C)	Percent COD Removal
				Influent	Effluent		
1	237	0.6	7.9	7.0	6.7	22	62
2	252	0.7	8.2	7.2	7.1	22	59
3	234	1.2	7.1	7.3	7.5	22	62
4	217	0.7	7.7	7.4	7.3	25	65
5	238	0.6	7.4	6.9	6.8	25	62
6	232	0.8	7.6	7.1	6.4	22	63
7	229	0.4	7.8	6.7	7.0	23	63
8	224	0.2	7.1	6.8	7.1	22	64
9	243	0.3	8.3	7.0	7.2	22	61
10	232	0.5	7.8	6.5	6.7	23	63
Average	234	0.6	7.7	6.9	6.9	23	62

Average Influent COD = 619.2 mg/l

Dissolved Solids Influent = 515.4 mg/l

disperse dye recovery. The technique involved also provided the necessary increase of concentration for liquid chromatography analysis. As seen in Table 11, the disperse dyes were recoverable at high percentages using one bed volume of benzene as the solvent.

Initial studies of acid dye recovery by column chromatography used methanol as the solvent. This system resulted in low recovery for some acid dyes as shown in Table 12. For this work 70% recovery was set as the minimum acceptable limit for dye recovery. Comparing this value to the data for methanol extractions in Table 12, four of the acid dyes failed to meet the minimum requirements.

After numerous studies, it was found that a pyridine:tetrahydrofuran:1% ammonium (40/40/20) mixture increased recovery of acid dyes to the minimum acceptable limit. Acid dye recoveries were improved with this solvent system significantly as shown in Table 12. Examples are Acid Yellow 19 which improved from 30% to 70% recovery and Acid Red 151 which improved to nearly complete recovery of 97%.

As stated earlier the benzene extraction of disperse dyes allowed air into the resin bed of the column. Extraction procedures for acid dyes followed the benzene elution. Although air was in the resin bed after the benzene extraction, this did not seriously affect the acid dye recovery. The lower recoveries were apparently due to the partition of the dyes between the resin and solvents.

C. Liquid Chromatography Analysis of Disperse Dyes

Percent removals of selected disperse dyes were determined by analysis of reactor influents and effluents by liquid chromatography.

TABLE 11

Disperse Dye Recovery by Column Chromatography

Disperse Dye	Percent Recovery
Yellow 23	77
Yellow 3	98
Yellow 54	77
Red 60	89
Red 55	95
Blue 7	73
Blue 120	83

TABLE 12

Acid Dye Recovery by Column Chromatography

Acid Dye	Methanol Extraction	Total
Blue 25	80	80
Blue 40	57	82*
Yellow 19	30	70*
Yellow 135	100	100
Yellow 151	75	75
Red 151	40	97*
Red 337	75	75
Orange 128	40	76*

*Total includes recovery by methanol plus the pyridine:tetrahydrofuran:ammonium hydroxide mixture.

Each disperse dye recovered from waste water samples could be separated and quantitated by using various tetrahydrofuran:cyclohexane solvent-mixtures.

The efficiency of the column-liquid chromatography was determined in order to calculate the actual amount of dye present in wastewater. It was evaluated by injecting a sample of the influent of the bench scale system recovered and concentrated by column chromatography into the liquid chromatograph. The data were recorded for each disperse dye at its respective instrument adjustments. The efficiency was then calculated by comparison of the actual concentration and the calculated concentration. The overall efficiencies of column-liquid chromatography for disperse dyes in this study were in the range of 65-92 percent (Table 13). These efficiencies were acceptable for the detection of disperse dyes at low concentrations in wastewater.

It was necessary to calibrate the liquid chromatograph to determine the unknown concentrations of the selected disperse dyes in wastewater samples. This calibration of the liquid chromatograph gave linear curves of absorption versus concentration for each disperse dye examined. This indicated that absorption was directly proportional to concentration, therefore the system could be used for quantitative purposes.

1. Disperse Blue Dyes

Initially the disperse blue dyes presented a serious problem in quantitative analysis by liquid chromatography. At that time the Micromeritics Model 780 with a deuterium lamp was used as the detector. With this detector only highly concentrated solutions of blue dyes could

TABLE 13

Column-Liquid Chromatography System
Efficiencies for Disperse Dyes

Disperse Dye	Dye in Effluent (mg/l)	Dye Detected (mg/l)	Percent Removal Efficiency
Yellow 54	5.76	4.05	70
Yellow 3	5.78	5.31	92
Yellow 23	8.21	5.44	66
Red 60	3.97	3.44	87
Red 55	1.82	1.27	70
Blue 7	1.82	1.18	65

be seen at 620 nm. Next, the Varian detector with a tungsten lamp was used. Although the dyes could be detected, the results were not reproducible. Finally a detector with a quartz-iodine lamp (Tracor) was used. The liquid chromatograph could be calibrated for Disperse Blue 7 using this detector. The instrument could not be calibrated with Disperse Blue 120 at concentrations less than 50 mg/1. These blue dyes were separated by liquid chromatography into a number of components. This may have also contributed to the problems encountered in their analysis.

In figure 11 the chromatograms of Blue 7 at concentrations of 5, 10, and 20 mg/1 are shown superimposed upon one another. A plot of absorbance versus concentration for each of the solutions is shown in figure 12. The curves of each major component in Blue 7 were linear, therefore studies were make including both compounds present.

The quantities of each component found in wastewater samples from the bench scale biooxidation system are listed in Table 14. The first component appearing on the chromatogram was removed with an efficiency of 65 percent. A removal of 98 percent of the second component indicated that this compound was more easily removed from wastewaters than the first component.

2. Disperse Red Dyes

Liquid chromatography was used in a similar manner to investigate the percent removal of Disperse Red 60 and Red 55. All the detectors were examined for their ability to detect red dyes at 520 nm. The Micromeritics instrument was sensitive to the red dyes only at concentrations on the order of 100 mg/1. Both the Varian and Tracor detectors were capable of detecting red dyes as they were eluted from the column. The Tracor

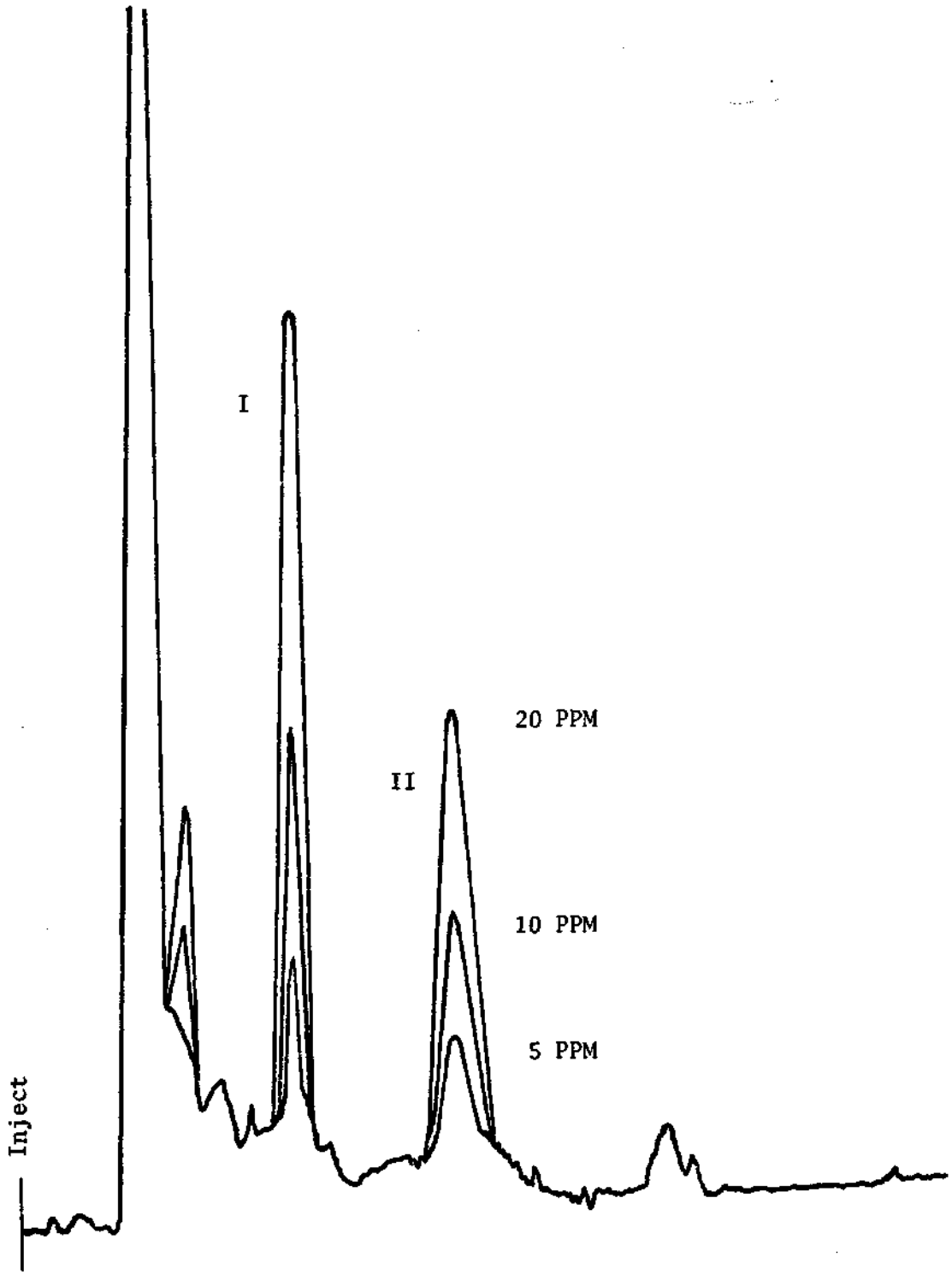


Figure 11. Liquid Chromatograms of Disperse Blue 7.

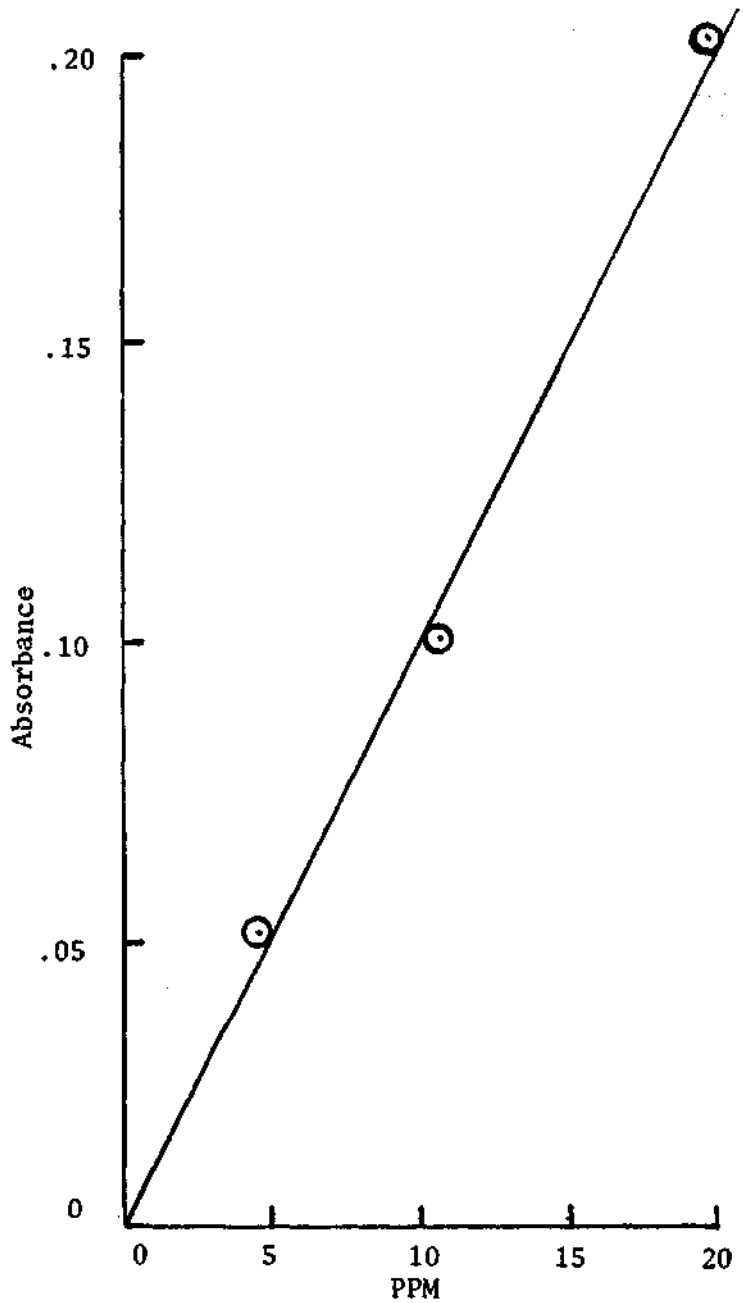


Figure 12. Disperse Blue 7 (Absorbance versus Concentration).

TABLE 14

Wastewater Samples of Disperse Blue 7

Sample	Component 1		Component 2	
	mg/l	Percent Removal	mg/l	Percent Removal
1	0.29	75	0.03	97
2	0.53	55	0.04	96
3	0.60	49	0.05	95
4	0.66	44	0.03	97
5	0.53	55	0.03	97
6	0.32	73	0.01	99
7	0.45	62	0.02	98
8	0.41	65	0.04	96
9	0.28	76	0.03	97
10	0.16	86	0.03	97
	Average	64		97

detector was chosen for investigating the red dyes since it could detect the red dyes in solutions at concentrations less than 5 mg/l.

Chromatograms of Disperse Red 55 at 5,10, and 20 mg/l are shown superimposed upon one another in figure 13. These absorbances are plotted versus concentration in figure 14. The linearity of this graph indicates that Disperse Red 55 obeys the Beer-Lambert Law.

The 10 samples from the bench scale biooxidation system were injected into the liquid chromatograph to study the percent removal of Red 55. The results are shown in Table 15. This dye was removed from wastewater with approximately 78 percent efficiency.

Liquid chromatograms of Disperse Red 60 are shown in figure 15. The absorbances were plotted with respect to their concentrations to establish a calibration curve for Red 60 as shown in figure 16. This curve was also linear with a slope of .01130.

Ten wastewater samples were injected to examine the percent removal of Red 60. In Table 16 the extent of removal is shown. The average removal efficiency was 59 percent. This was not as high a percentage as Red 55 or the Yellow dyes indicating that Red 60 was more resistant to removal than the other dyes.

3. Disperse Yellow Dyes

Disperse Yellow 3, Yellow 23, and Yellow 54 were investigated using the Varian detector with a tungsten lamp. Using this detector and a solvent gradient mode of operation, each of the yellow dyes could be quantitated by a single injection. The liquid chromatograph was calibrated as previously described.

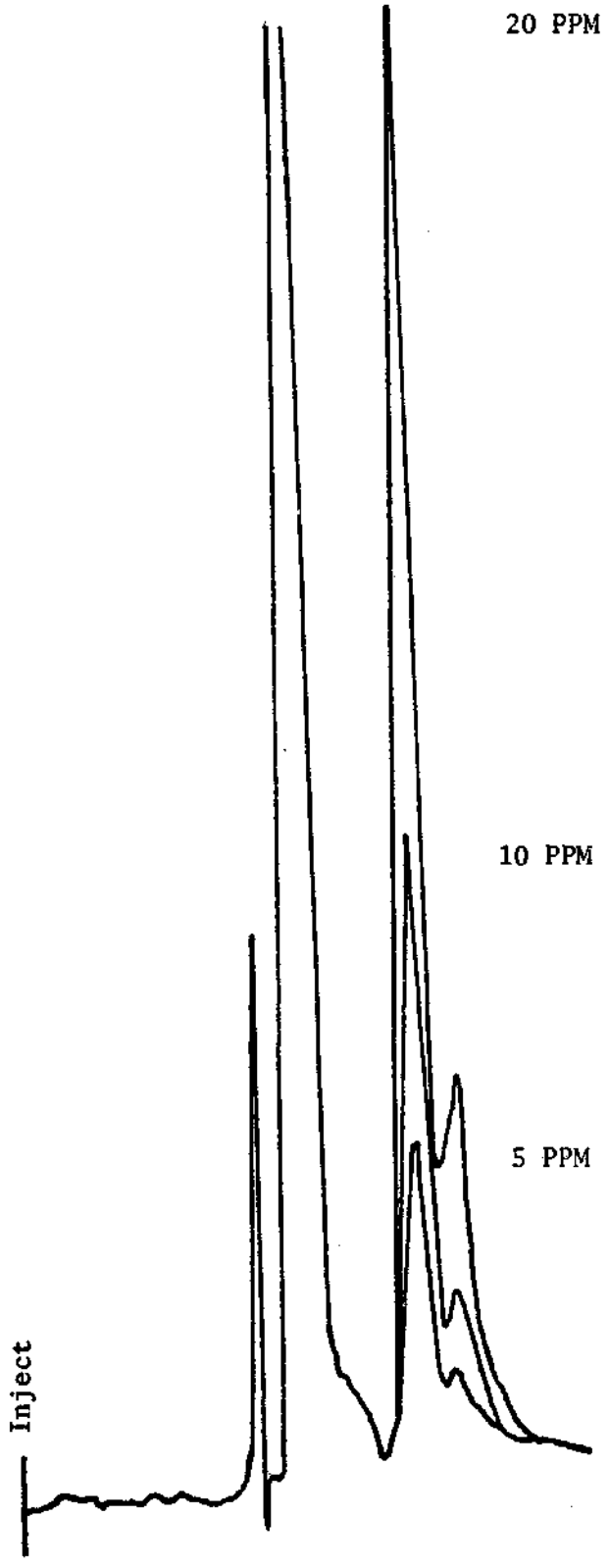


Figure 13. Liquid Chromatograms of Disperse Red 55.

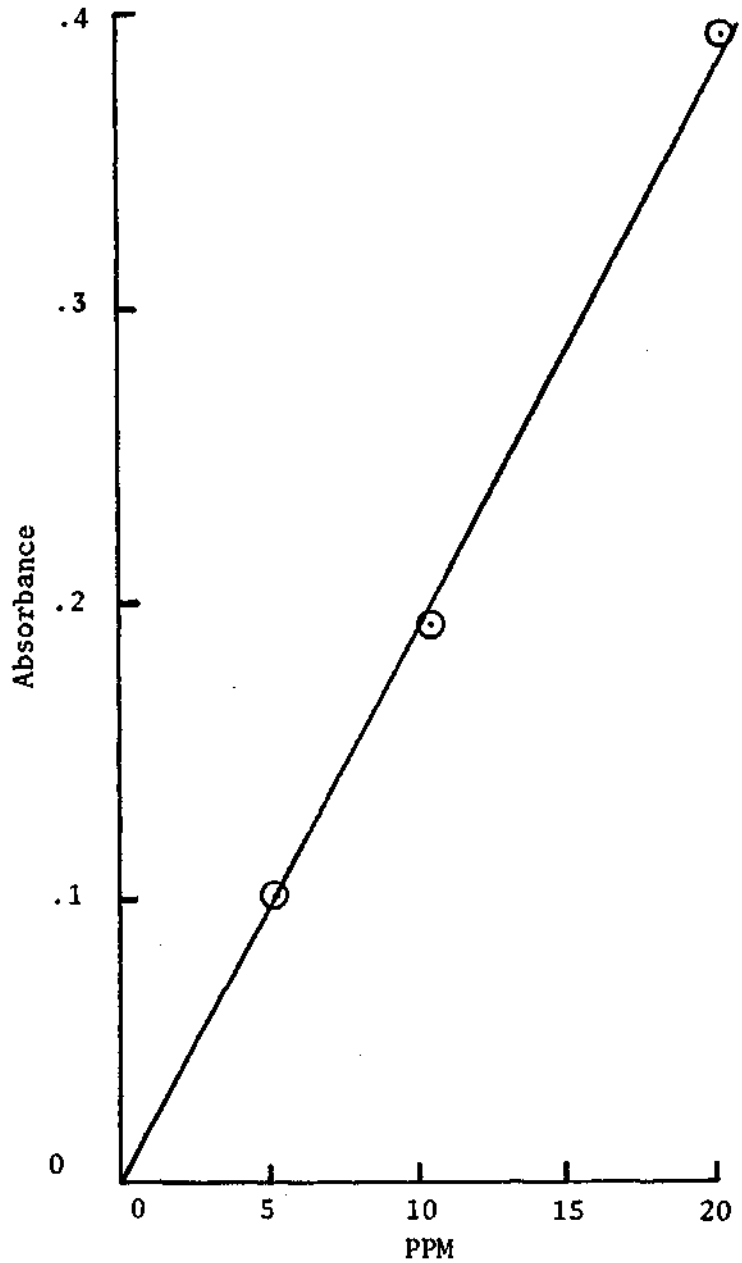


Figure 14. Disperse Red 55 (Absorbance versus Concentration).

TABLE 15

Percent Removal of Disperse Red 55

Sample	mg/l	Percent Removal
1	0.23	82
2	0.32	75
3	0.29	77
4	0.24	81
5	0.11	91
6	0.22	83
7	0.19	85
8	0.29	77
9	0.24	81
10	0.62	51
	Average	78

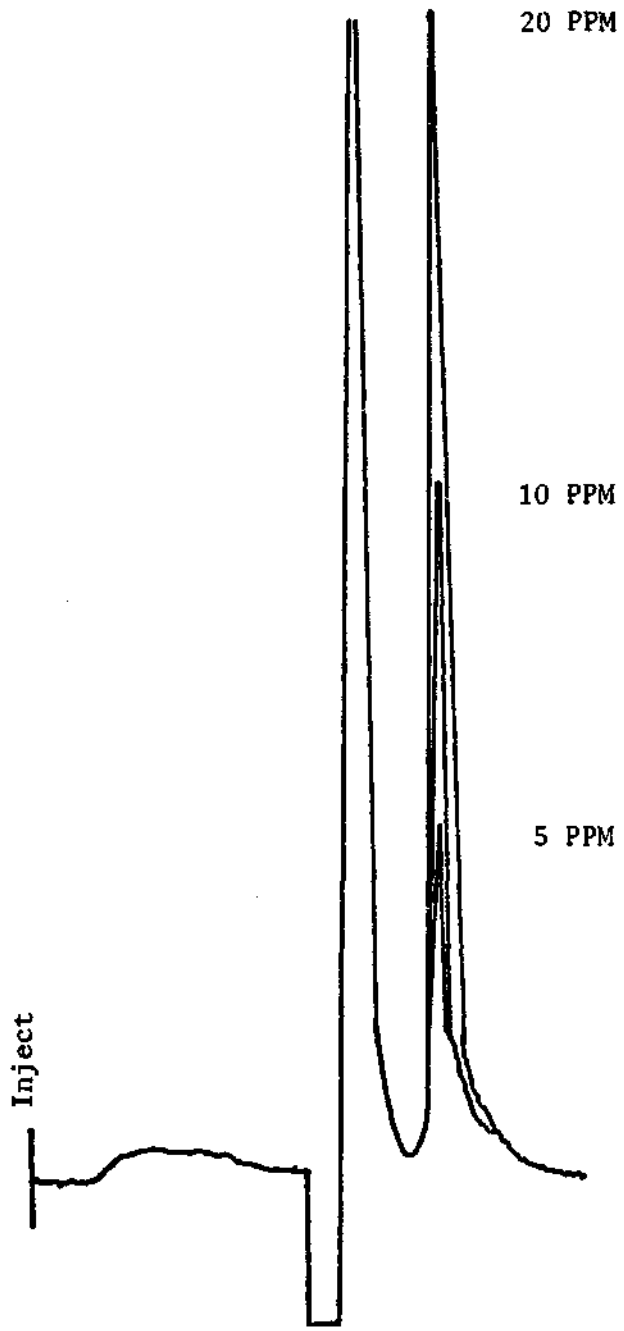


Figure 15. Disperse Red 60 - Liquid Chromatography.

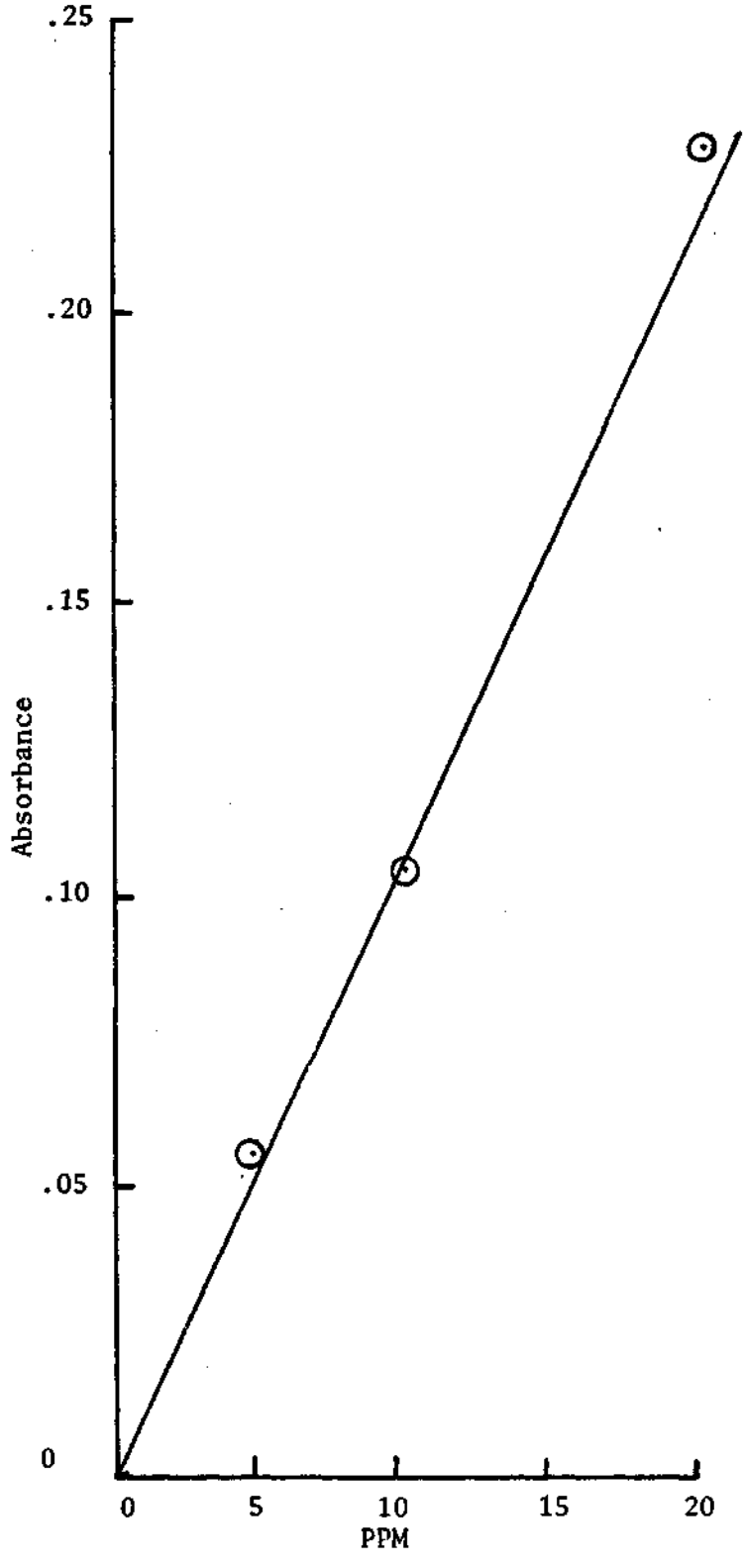


Figure 16. Disperse Red 60 - Calibration Curve.

TABLE 16

Percent Removal of Disperse Red 60

Sample	mg/l	Percent Removal
1	2.46	28
2	2.77	19
3	1.48	57
4	1.33	61
5	.88	74
6	.48	86
7	1.42	59
8	1.22	65
9	1.06	69
10	.84	76
	Average	59

Two solutions containing each of the yellow dyes at 5 and 10 mg/l were injected into the liquid chromatograph. The chromatograms of these injections are shown superimposed in figure 17. Plotted in figure 18 are the calibration curves of absorbance versus concentration for each yellow dye. As can be seen these graphs were also linear.

The wastewater samples from the bench scale biooxidation system were examined for yellow dye content. Absorbance peaks from the dyes were identified by retention times.

In Table 17 the removal efficiencies of Disperse Yellow 23 are listed. The average 66 percent removal was the lowest of the yellow dyes examined. As seen in Table 18, the average removal of Yellow 3 is 93 percent, which was the highest removal efficiency of the yellow dyes. Yellow 54 was intermediate between Yellow 3 and Yellow 23 with an average of 77% removal as shown in Table 19. Removal of these dyes did not appear to be directly related to the basic structure of the dyes. Since Yellow 3 and Yellow 23 were monoazo and diazo dyes respectively, the elimination of these dyes may have been a function of substituent groups.

In the chromatograms of the effluent samples a number of small peaks appeared which were not present in the chromatogram of the influent. Therefore they were probably products of biodegradation of the dyes.

D. Acid Dye Analysis by Liquid Chromatography

Selected acid dyes were also studied by liquid chromatography. Each acid dye could be separated using the paired ion technique. The liquid chromatograph could be calibrated for each acid dye.

The efficiency of the column-liquid chromatography was investigated

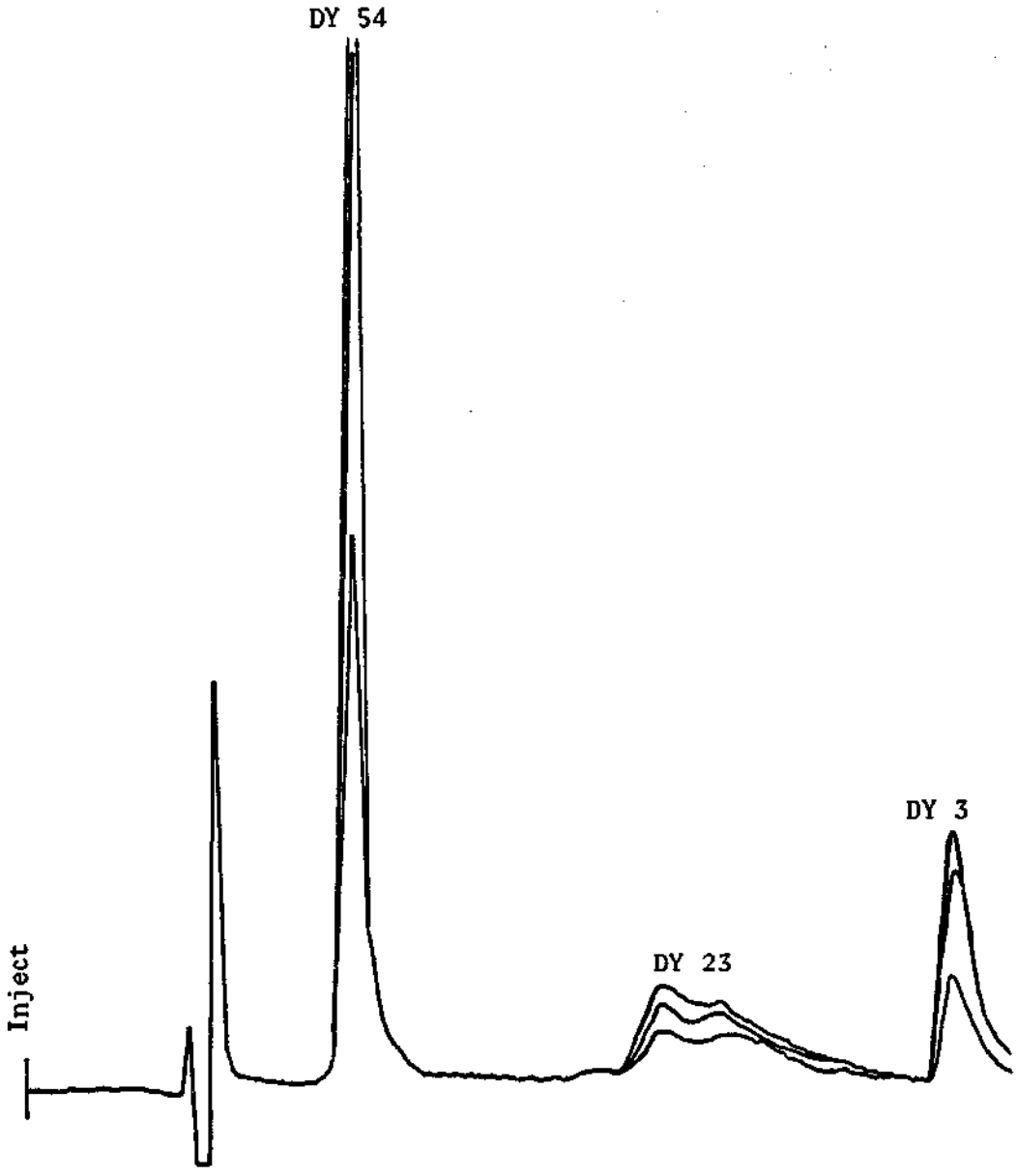


Figure 17. Liquid Chromatograms of the Disperse Yellow Dyes.

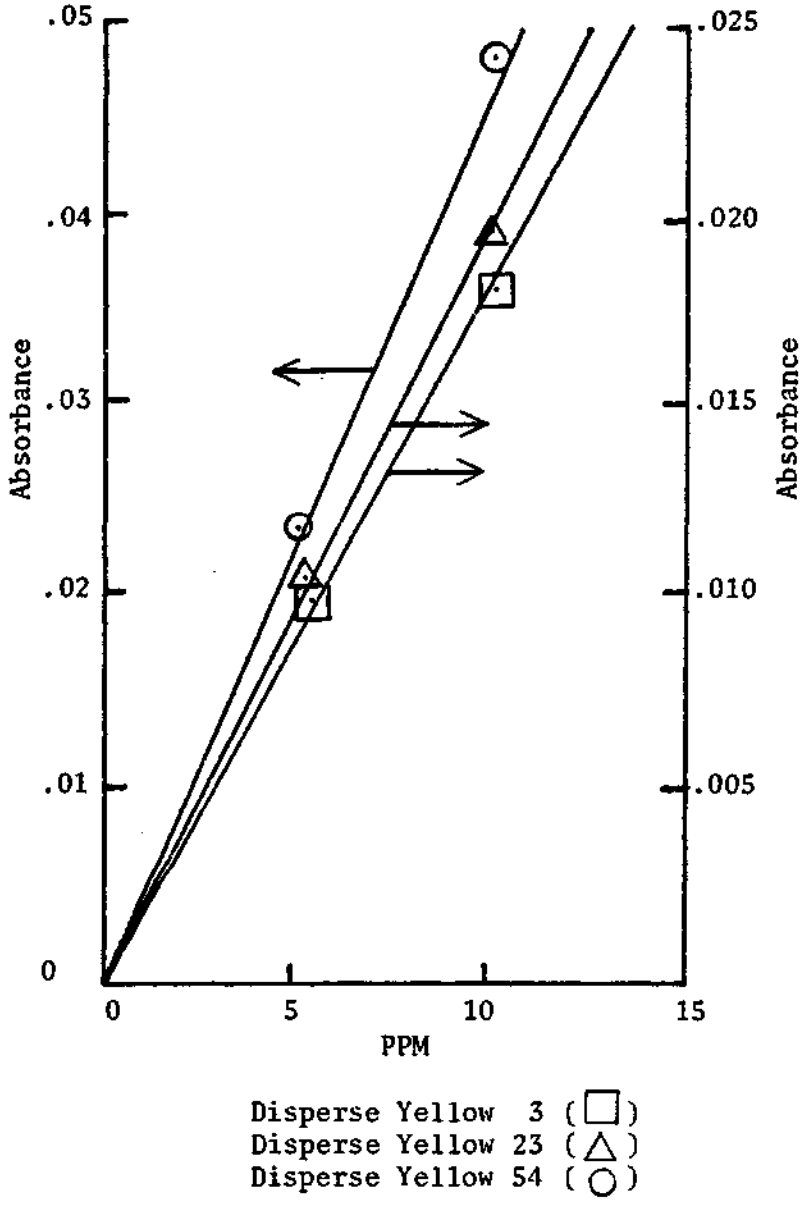


Figure 18. Calibration Curves of Disperse Yellow Dyes.

TABLE 17

Disperse Yellow 23 - Percent Removal

Sample	mg/l	Percent Removal
1	1.63	70
2	1.85	66
3	2.34	57
4	1.79	67
5	2.28	58
6	1.90	65
7	1.47	73
8	1.74	68
9	1.52	72
10	2.12	61
	Average	66

TABLE 18

Percent Removal of Disperse Yellow 3

Sample	mg/l	Percent Removal
1	0.95	82
2	0.53	90
3	0.37	93
4	0.37	93
5	0.21	96
6	0.16	97
7	0.22	96
8	0.32	94
9	0.16	97
10	0.32	94
	Average	93

TABLE 19

Percent Removal of Disperse Yellow 54

Sample	mg/l	Percent Removal
1	0.98	76
2	1.45	64
3	0.74	82
4	0.73	82
5	0.68	83
6	0.96	76
7	0.73	82
8	1.01	75
9	0.88	75
10	1.03	75
	Average	77

in order to calculate the actual amount of dye present in wastewater. These data were obtained by injecting samples of the bench scale influent recovered and concentrated by column chromatography into the liquid chromatograph. The efficiencies were determined by comparing the actual concentrations present with the calculated concentrations. The efficiencies of column-liquid chromatography for each dye investigated are shown in Table 20. The results in this table appear to be low, and unacceptable. Further work is needed to develop a technique for efficient acid dye analysis.

The actual percent removal were not determined due to the analytical procedure for acid dyes. Although the actual percentages were not determined, the removal of acid dyes appeared to be low. This was determined from the intense color of several acid dye samples recovered by column chromatography from the bench scale effluent.

E. Wastewater Samples from Dalton

Samples taken from the Dalton waste treatment facility and the stream into which it discharges were examined by liquid chromatography to determine the presence of the dyes investigated in this research. The sample sources and concentrations of the dyes found are listed in Table 21. The quantities of the dyes found were higher than those found in the bench scale system. This was expected, since an average value was used in the bench scale work. The samples were taken on different days. The components of Disperse Blue 7 produced unexpected results. The second component was expected to undergo nearly 100 percent degradation, but as shown in Table 21 it appears in higher concentration than the first component.

TABLE 20

Column-Liquid Chromatography Efficiency of Acid Dyes

Dye	Efficiency (%)
Acid Yellow 19	10
Acid Yellow 135	0
Acid Yellow 151	18
Acid Red 151	13
Acid Red 337	12
Acid Blue 25	0
Acid Blue 40	24
Acid Orange 128	43

TABLE 21

Dalton Wastewater Samples

Sample Collection Point	Dye	Concentration (mg/l)
Clarifier	Disperse Red 55	3.3
	Acid Blue 25	0.1
	Acid Blue 40	0.1
Treatment System Effluent	Disperse Red 55	1.9
	Disperse Red 60	3.4
	Disperse Blue 7, Component 1	0.6
	Disperse Blue 7, Component 2	0.9
	Disperse Yellow 54	2.3

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

In comparing experimental results from this study with data from the Dalton wastetreatment facility two correlations were found. First, the influent and effluent of the bench scale unit had pH's of 6.9 which was similar to 6.8 for each flow at the Dalton facility (Tables 3 and 10). Secondly, the bench scale influent COD value of 619 mg/l was within 13 percent of the 546 mg/l COD value recorded for the Dalton system.

Although these similarities existed, there were two major differences. One, the dissolved solids concentration of the bench scale model (515 mg/l) was considerably lower than the 918 mg/l figure for the Dalton operation. This was due to the equation used to calculate dissolved solids (Chapter 3, Equation 1). The equation was designed to calculate dissloved solids concentrations in the range of typical wastewaters, not necessarily carpet manufacturing wastewaters. The second difference was the percentage of COD removed by biological activity. The COD removal efficiency of the bench scale system was 62 percent while 80 percent was recorded for the Dalton operation. This difference was probably due to the long term operating efficiency established by the Dalton treatment facility.

Additional studies on the biodegradation of carpet processing chemicals should encompass at least two major areas. One area for further research is the effect of retention time including sludge recycle on the

biodegradation of these processing compounds. The second area of interest is the response of activated sludge treating carpet processing chemicals to increases and decreases in the percentage of domestic waste.

Column chromatography was an excellent technique for the recovery of acid and disperse dyes. Its value lies in its ability to recover trace quantities of dyes from large volumes of wastewater, followed by concentration of the dyes for analysis. By selection of the proper solvent-resin system the technique could be extended for the recovery of many of the organic compounds present in carpet and textile wastewaters including the various classes of dyes.

Liquid chromatography was an excellent method for the separation and quantitations of various classes of dyes in solution. This technique can be extended to study a wide variety of compounds present in carpet production wastewater. The usefulness of this instrument stems from its ease of operation, good reproducibility and ability to yield quantitative analysis of complex mixtures.

The disperse dyes investigated were removed from wastewater. The removal may have occurred by dye adsorption on the sludge rather than biodegradation due to the low water solubility of disperse dyes. In additional studies extraction procedures should be developed to remove disperse dyes from sludge to elucidate the mechanism of dye removal from wastewater.

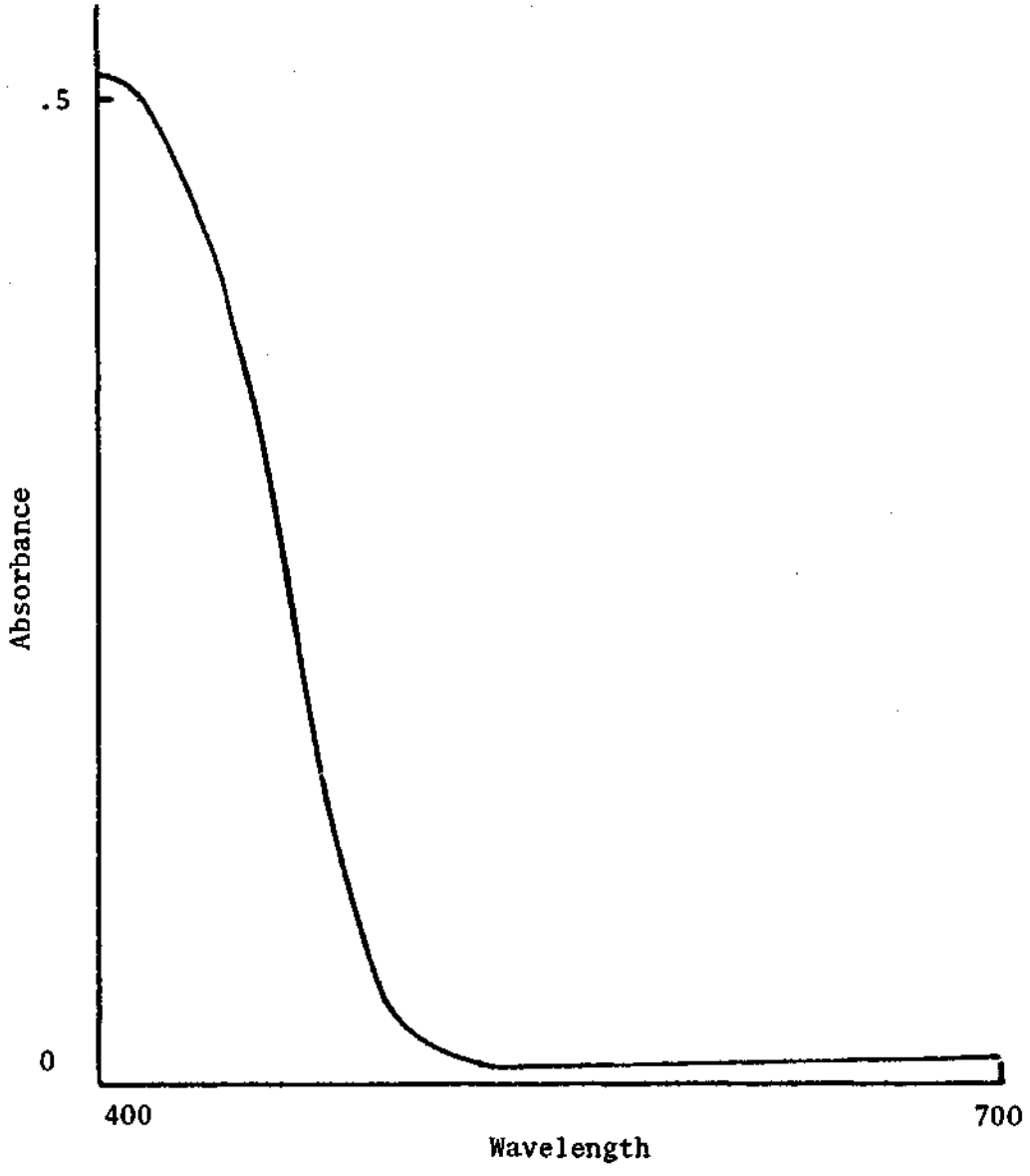
The percent removals of acid dyes were not determined using the techniques presented in this study. Although column chromatographic results were excellent, the results from liquid chromatography were not useful. The calibration curves (absorbance versus concentration)

from liquid chromatography were almost horizontal for most of the acid dyes making quantitation difficult. This was believed to be due to base line shifts in the gradient mode of operation. Future studies should be made to develop a technique to account for base line shifts, so that the biodegradation of the acid dyes can be investigated.

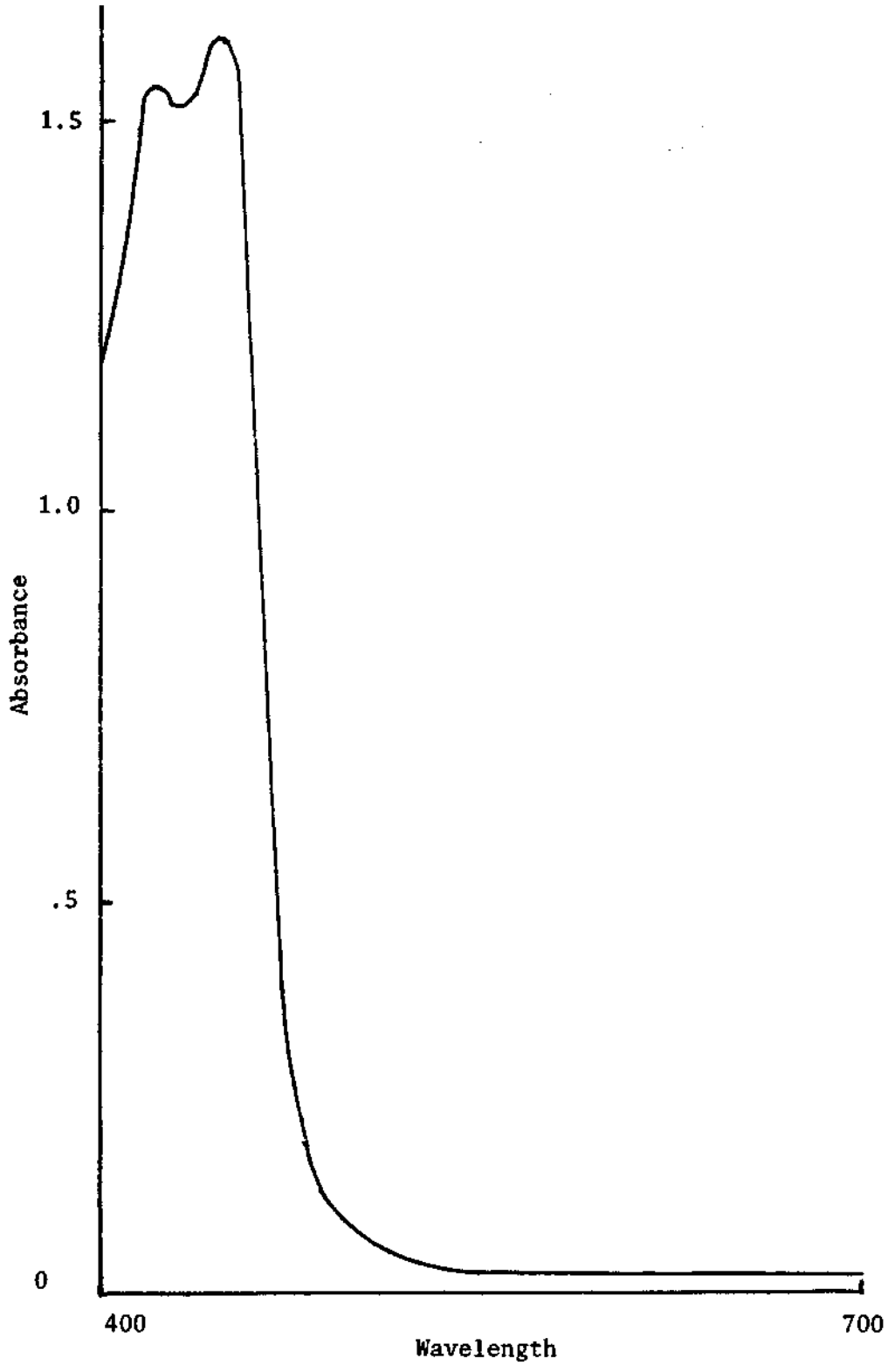
The procedures outlined in this research can be modified to investigate numerous classes of chemical compounds. They could be extended to include other classes of dyes used in carpet and textile processing. Following an investigation of the biodegradation of different dye classes, a study of the derivatives resulting from microbial metabolism would be of vital importance. The investigation of these derivatives is important in determining the pollutants actually being discharged into streams and waterways. Techniques similar to those developed in this study should be useful for the degradation products.

APPENDICES

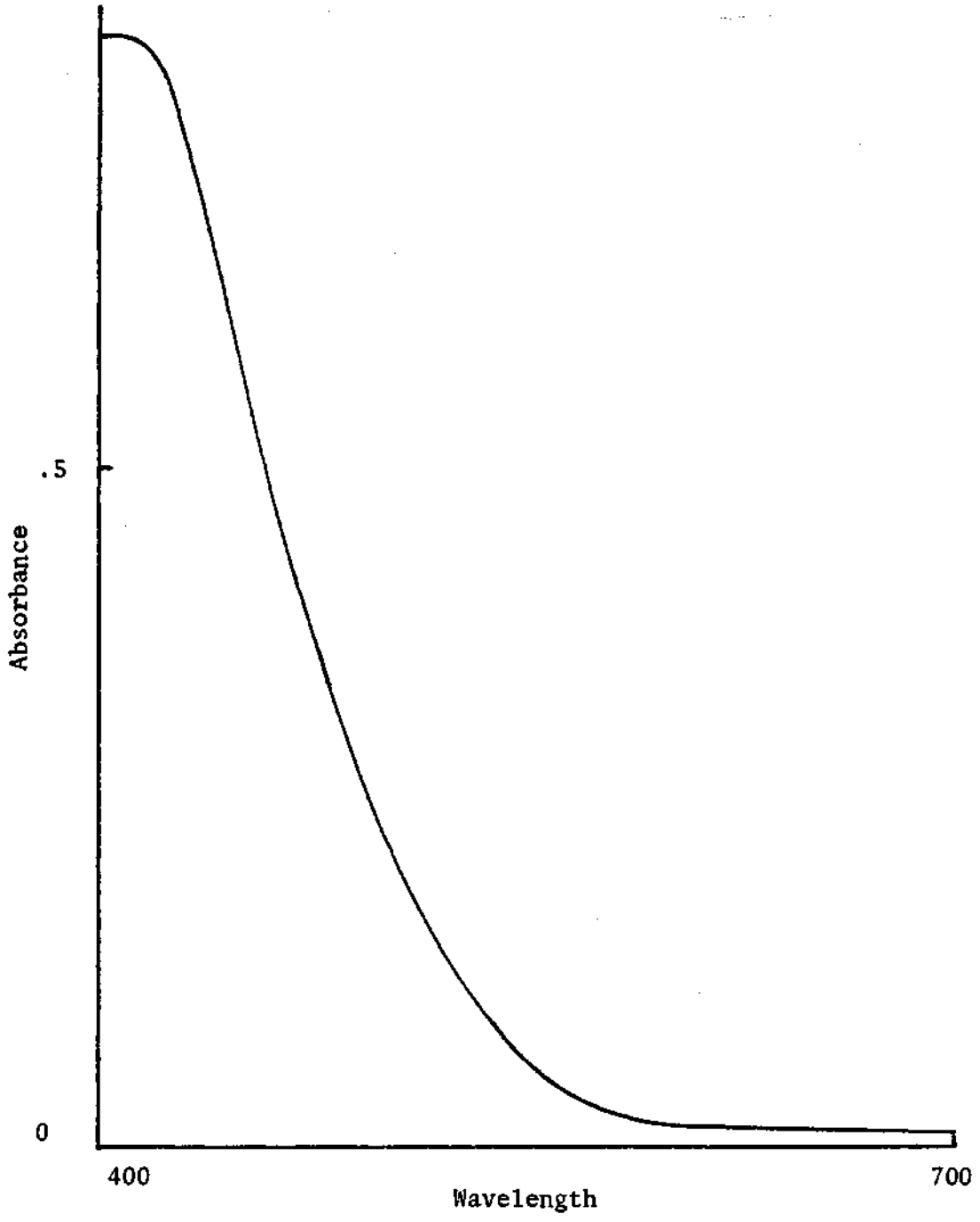
APPENDIX A



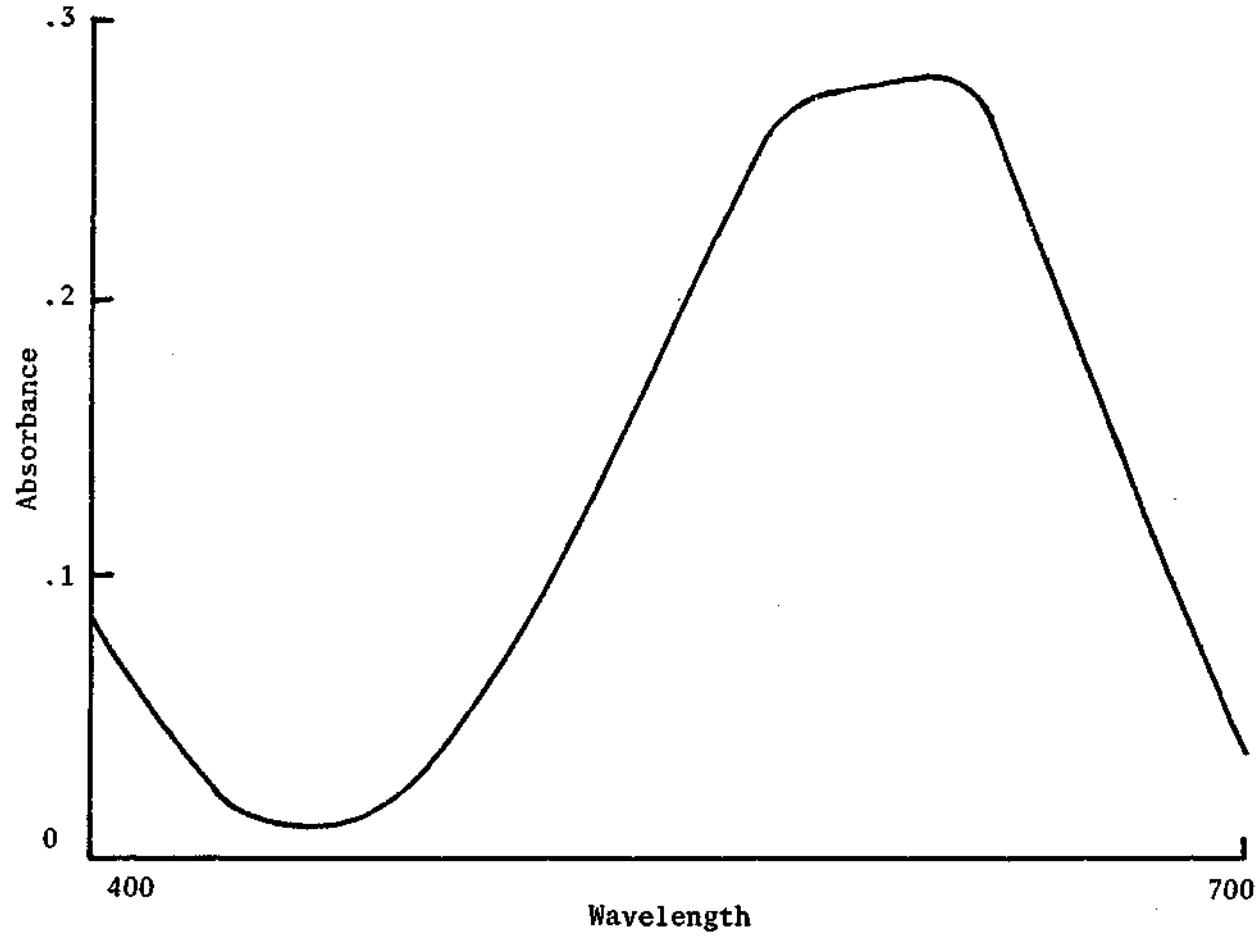
Absorbance Spectrum of Disperse Yellow 3 (10 PPM)



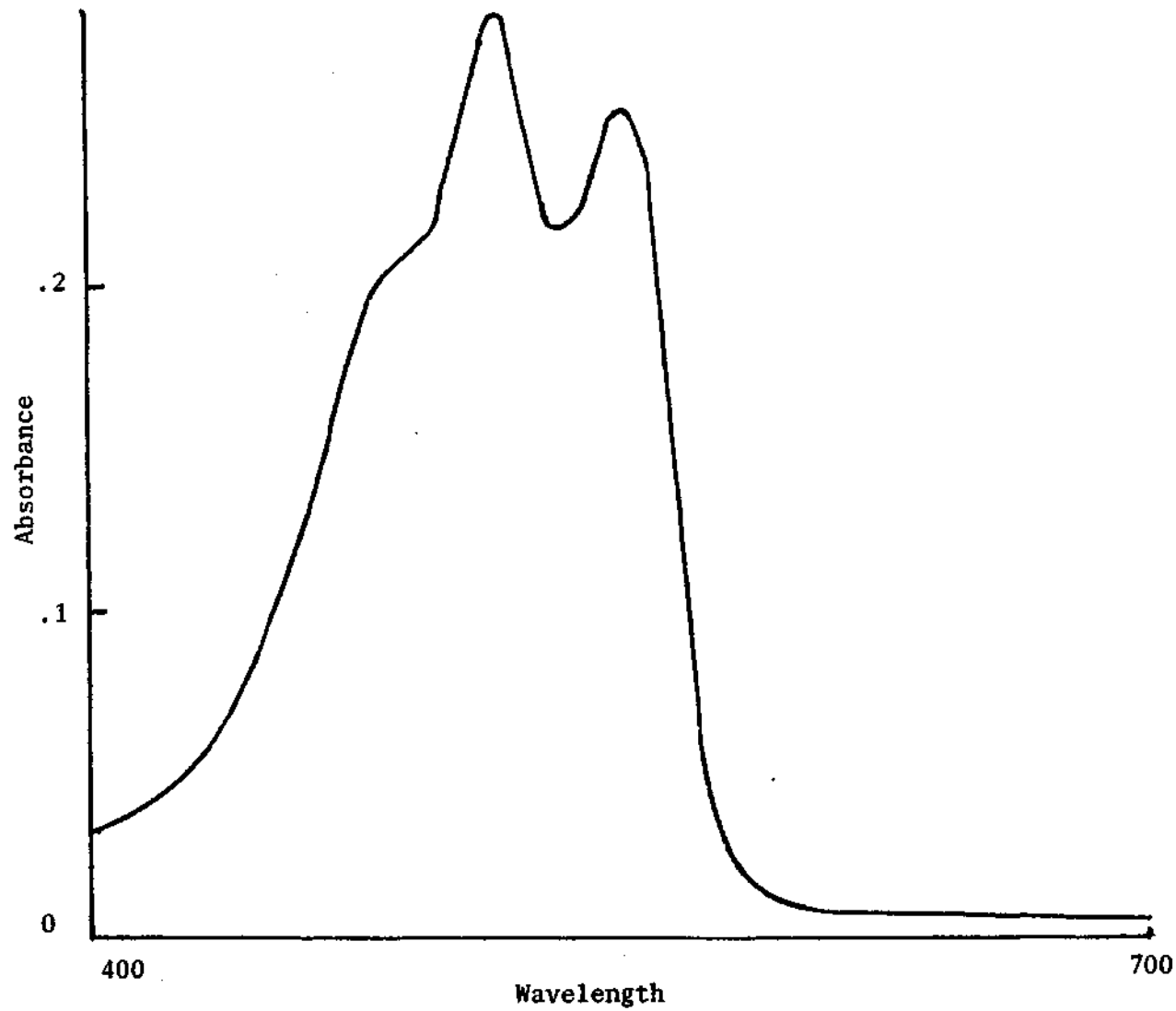
Absorbance Spectrum of Disperse Yellow 54 (10 PPM)



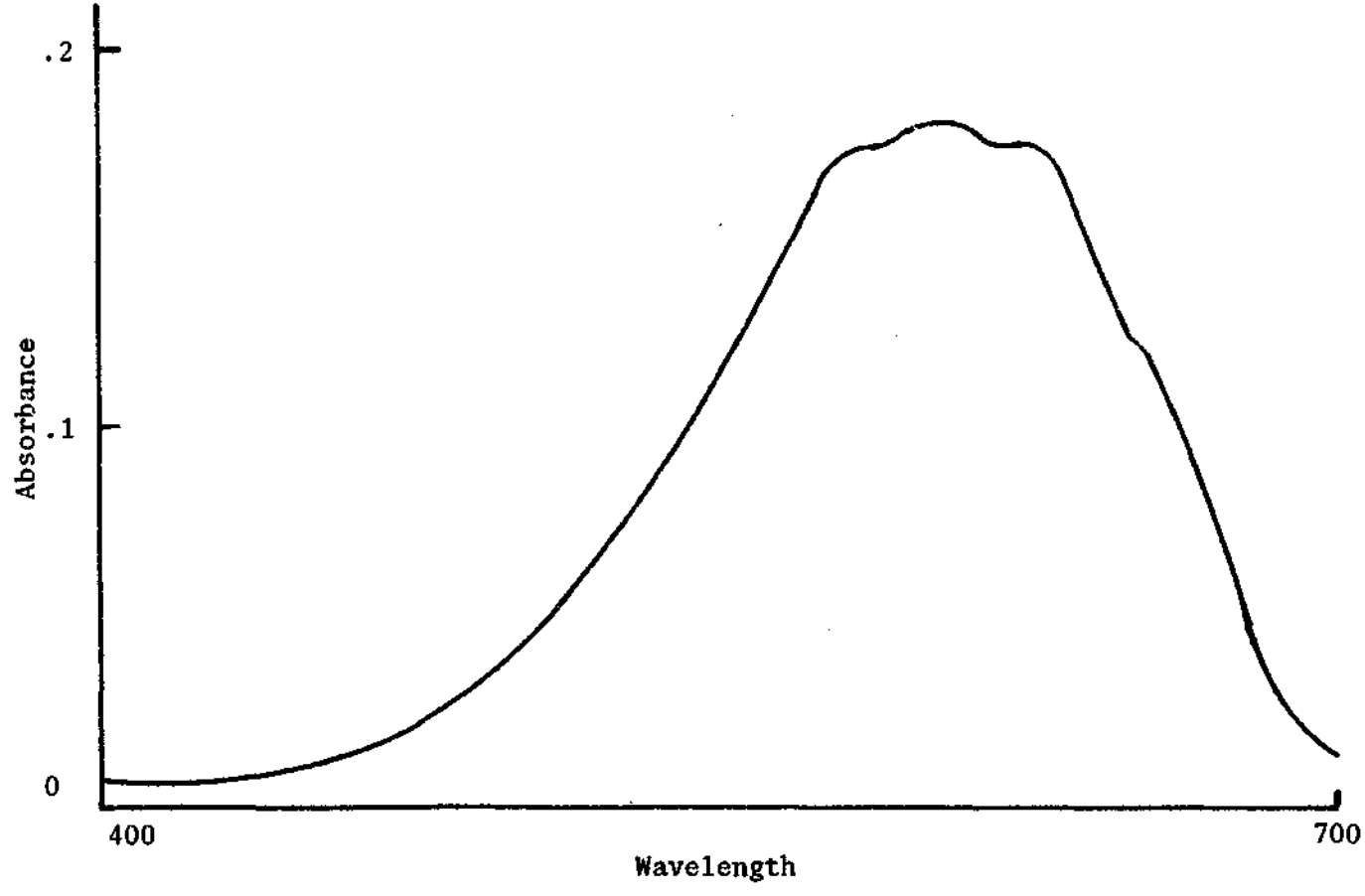
Absorbance Spectrum of Disperse Yellow 23 (10 PPM)



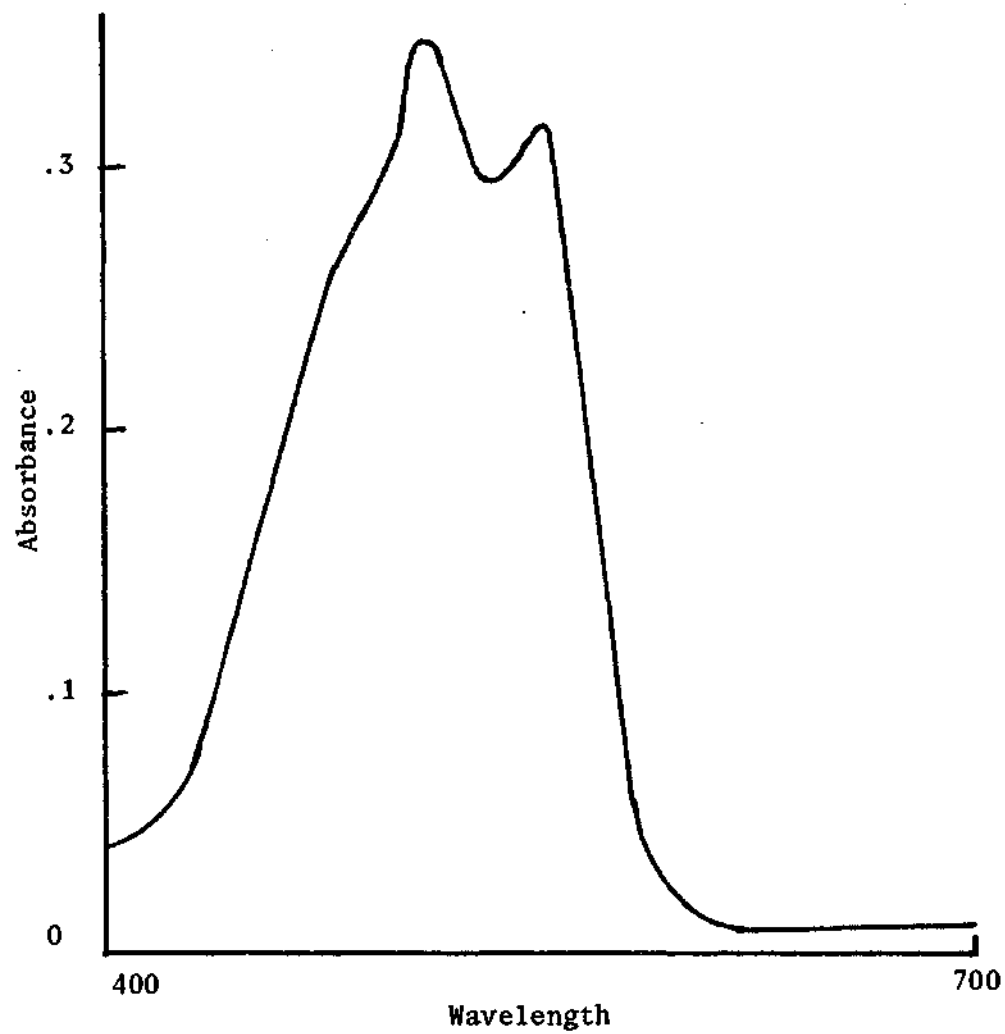
Absorbance Spectrum of Disperse Blue 120 (10 PPM)



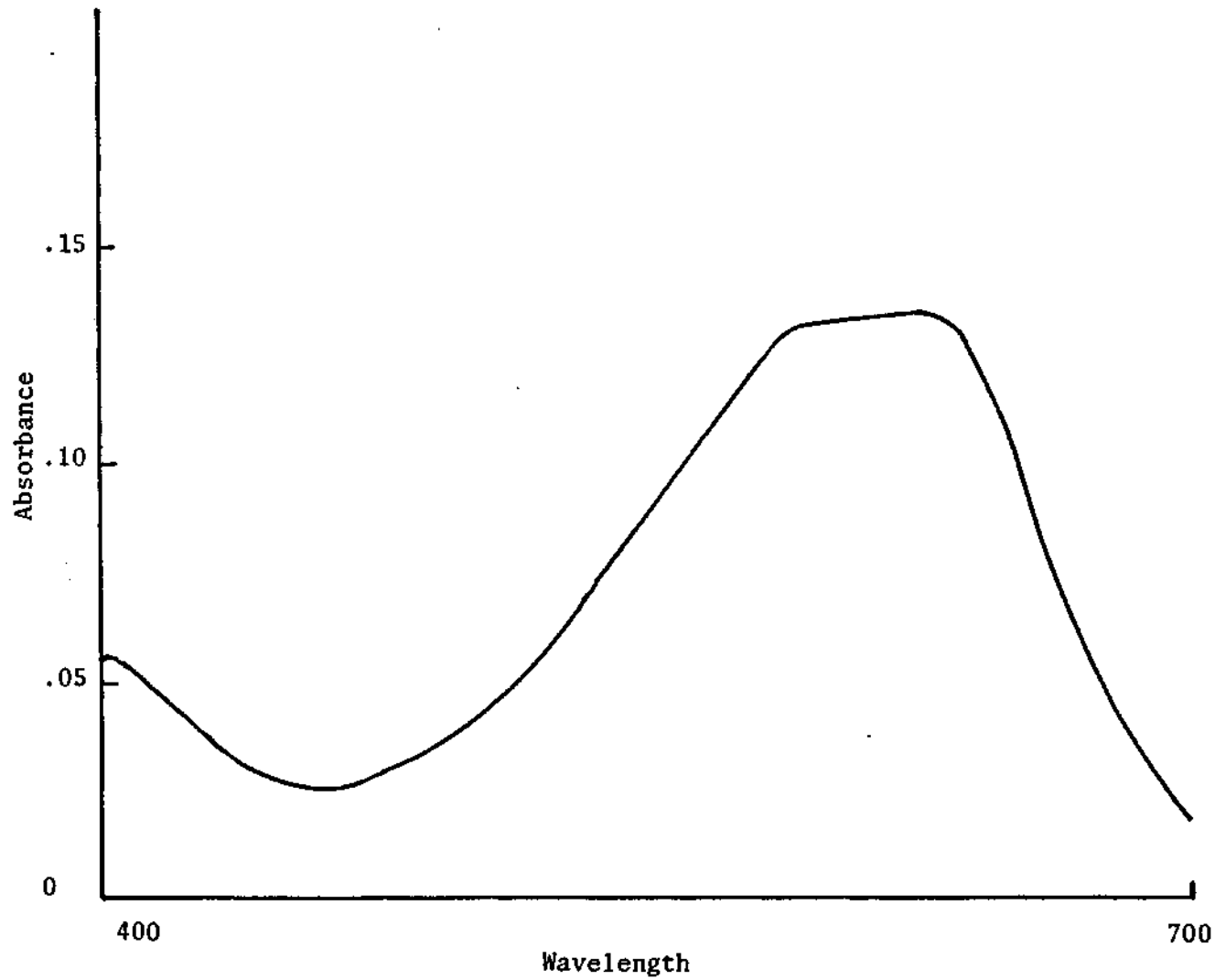
Absorbance Spectrum of Disperse Red 55 (10 PPM)



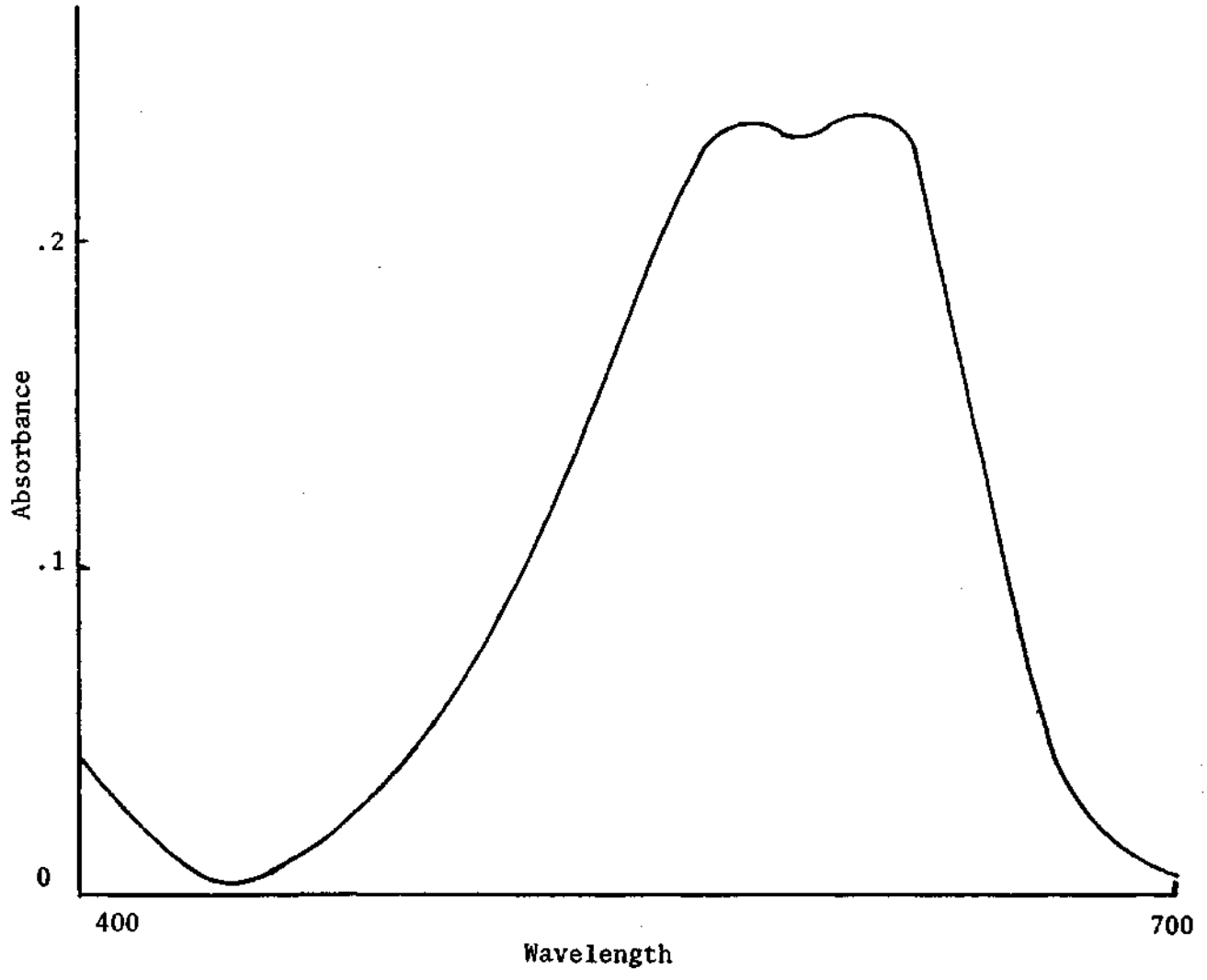
Absorbance Spectrum of Disperse Blue 7 (10 PPM)



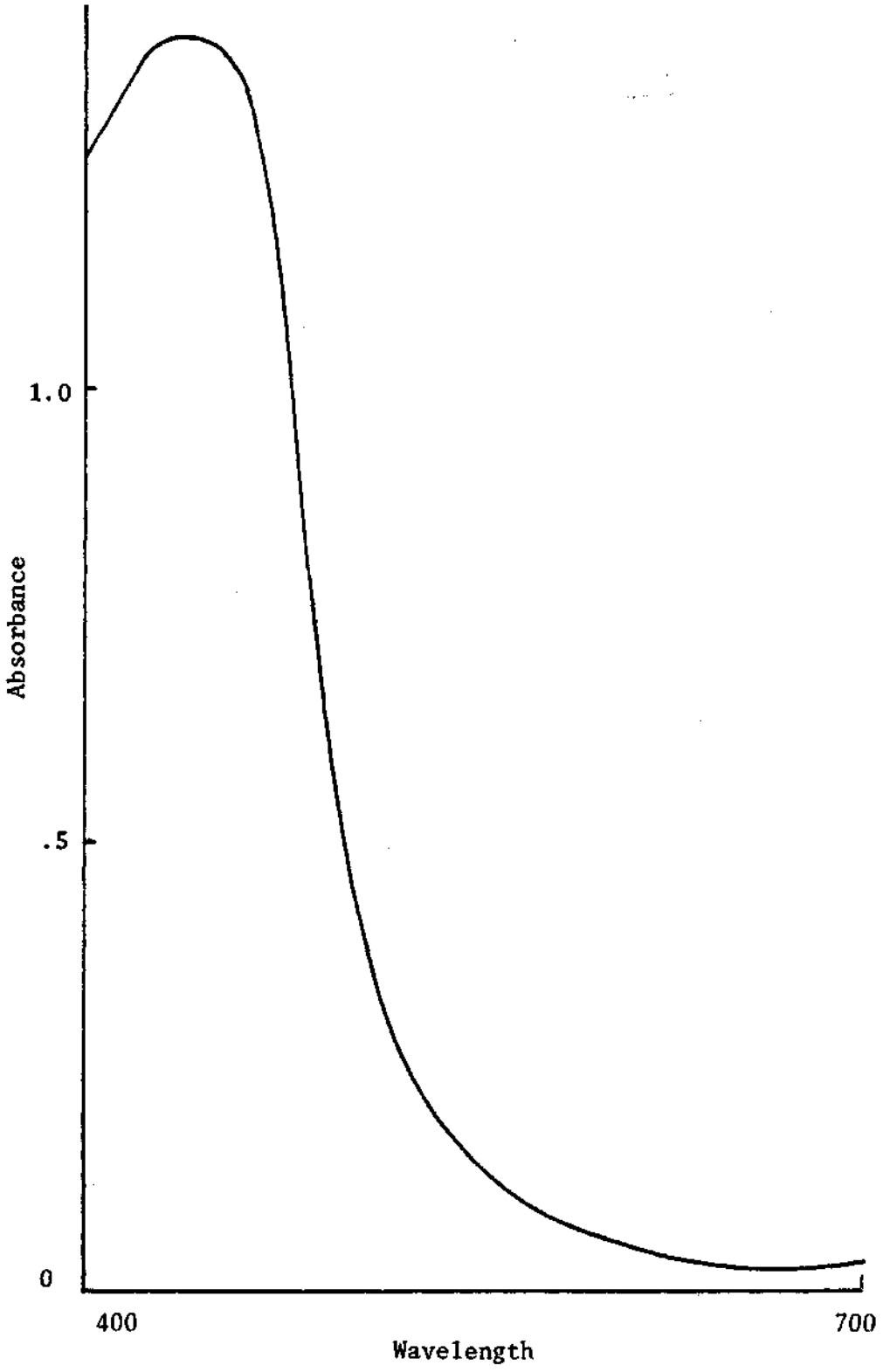
Absorbance Spectrum of Disperse Red 60 (10 PPM)



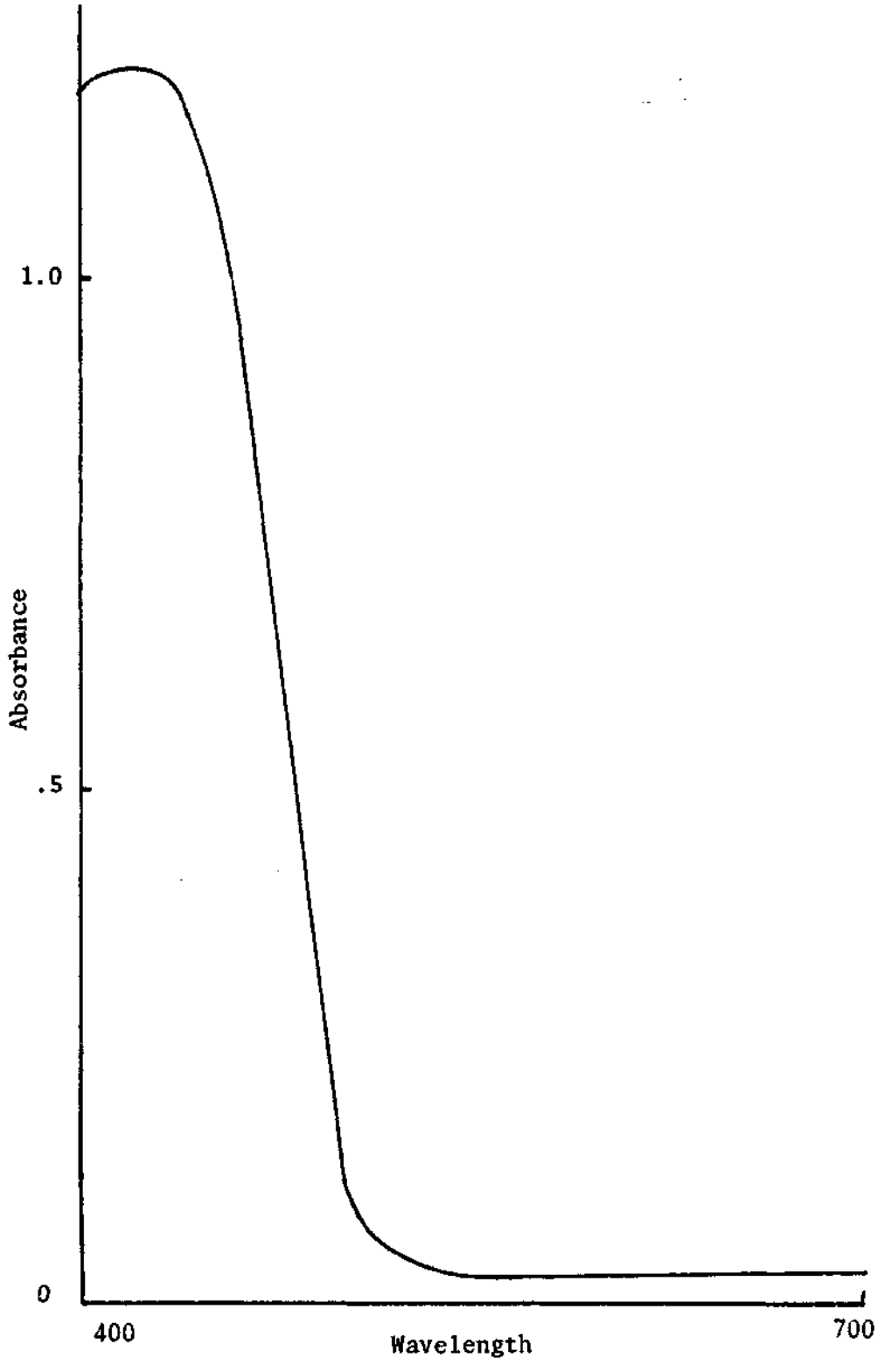
Absorbance Spectrum of Acid Blue 40 (10 PPM)



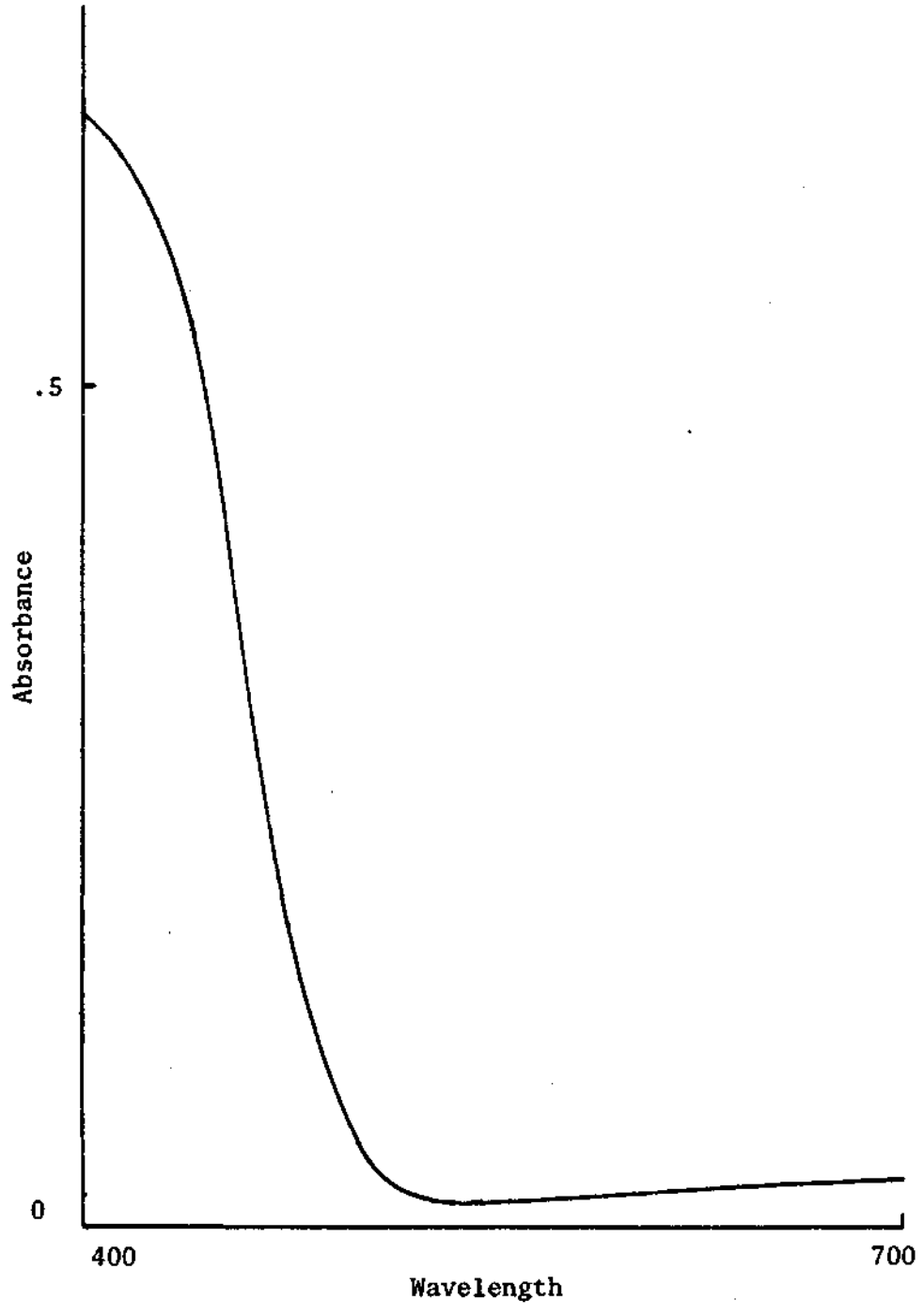
Absorbance Spectrum of Acid Blue 25 (10 PPM)



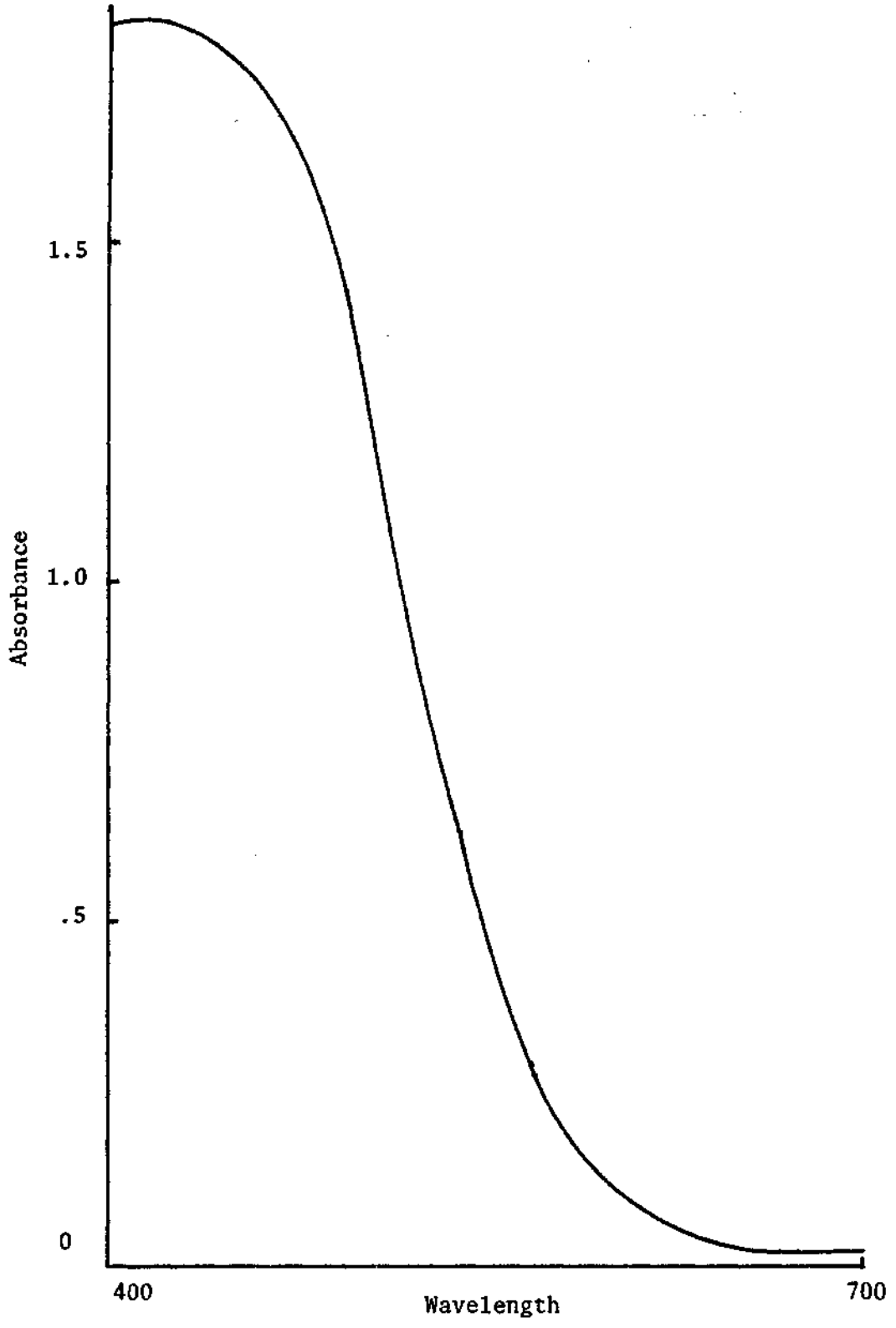
Absorbance Spectrum of Acid Yellow 151 (5 PPM)



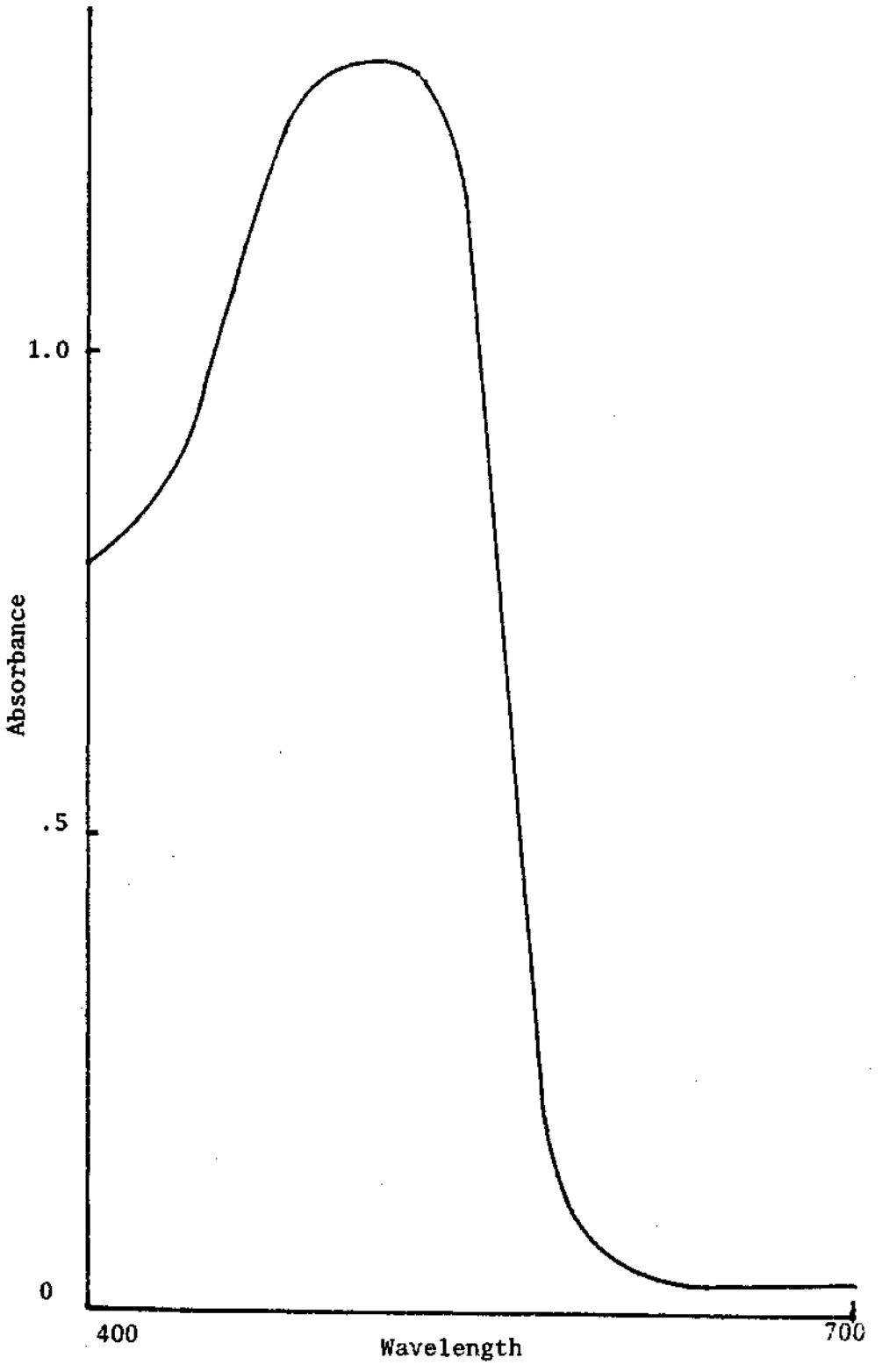
Absorbance Spectrum of Acid Yellow 19 (5 PPM)



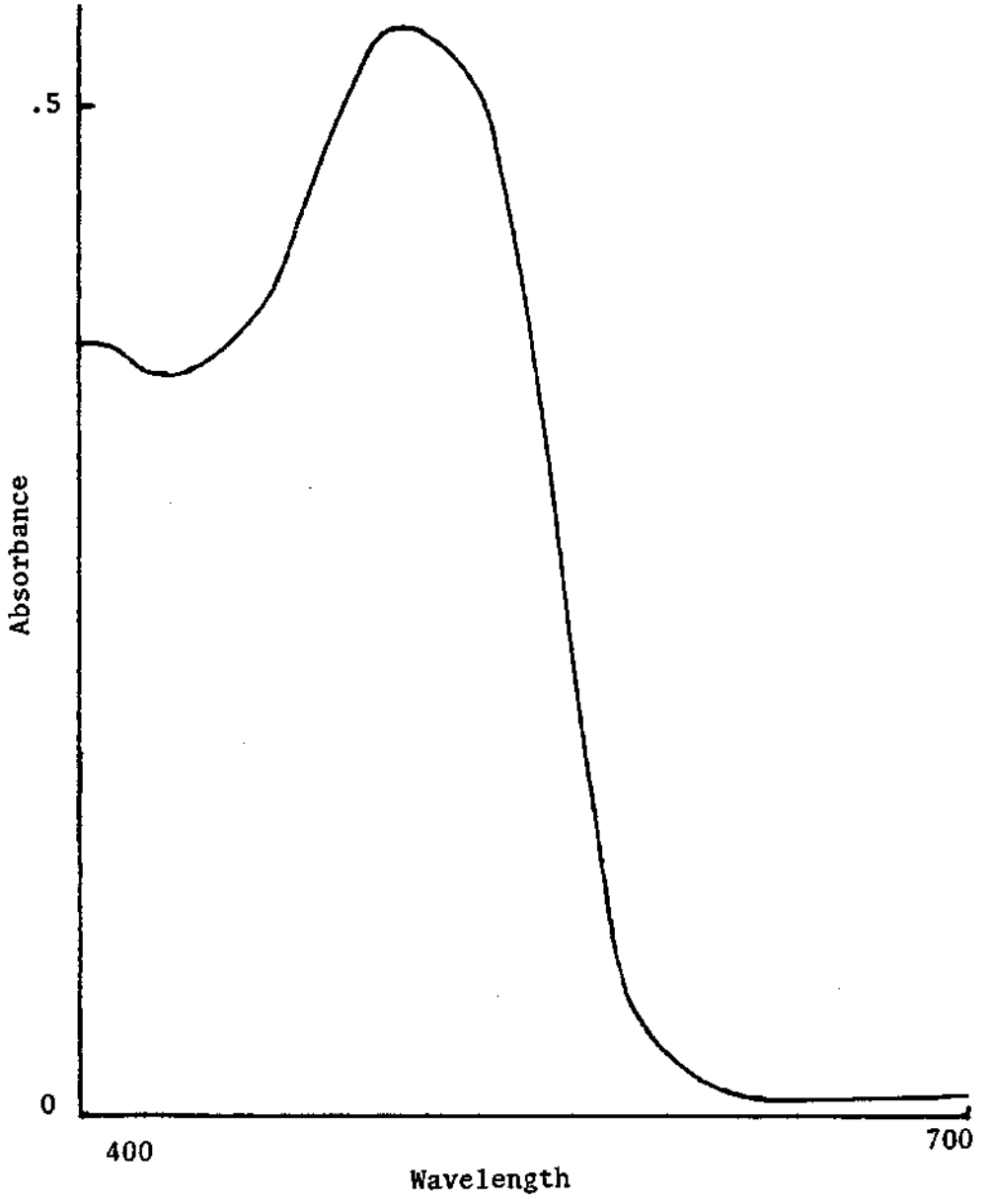
Absorbance Spectrum of Acid Yellow 135 (5 PPM)



Absorbance Spectrum of Acid Orange 128 (5 PPM)



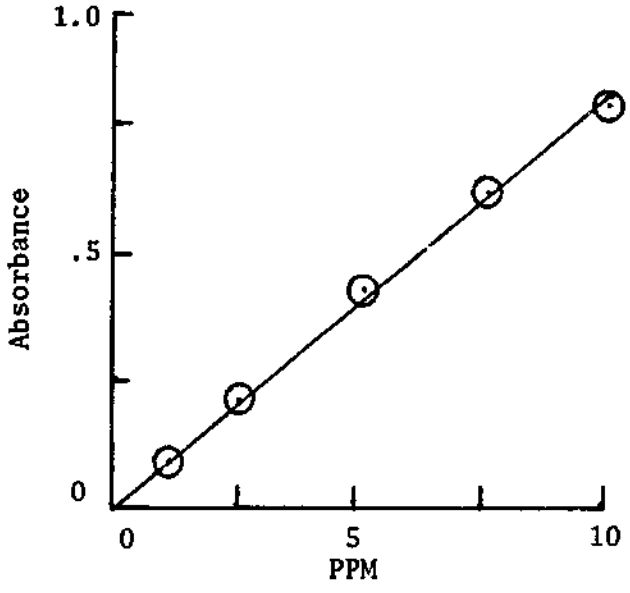
Absorbance Spectrum of Acid Red 337 (5 PPM)



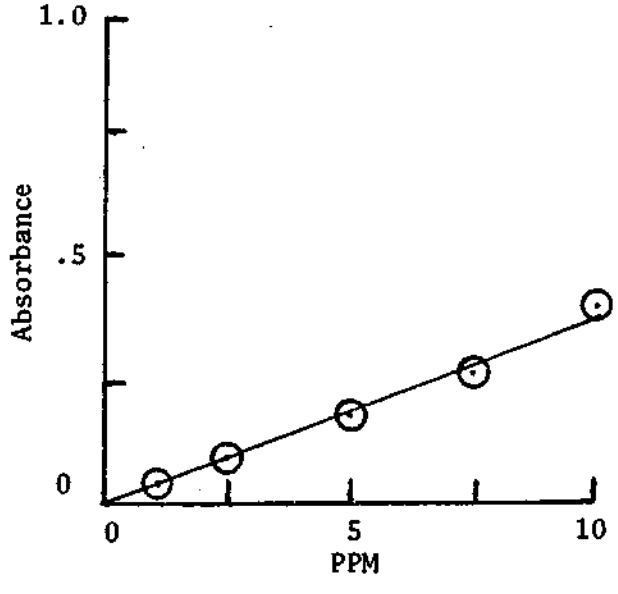
Absorbance Spectrum of Acid Red 151 (5 PPM)

APPENDIX B

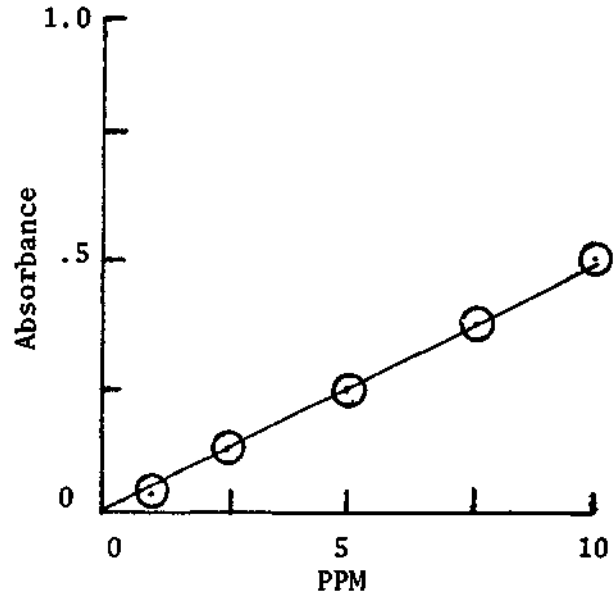
Concentration versus Absorption



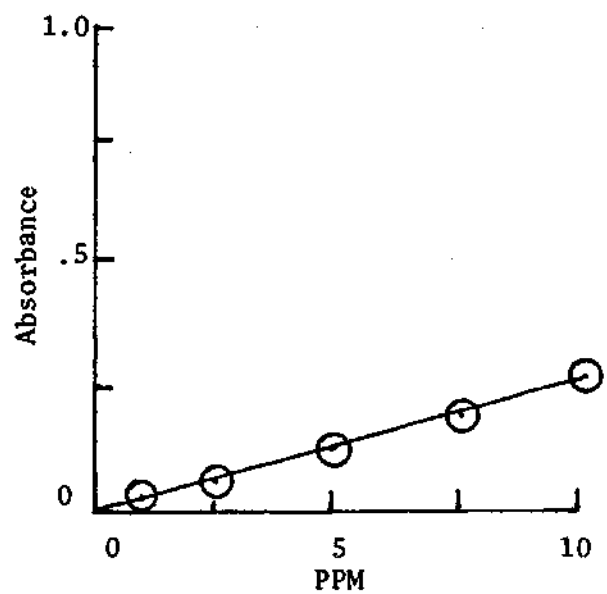
Disperse Yellow 23



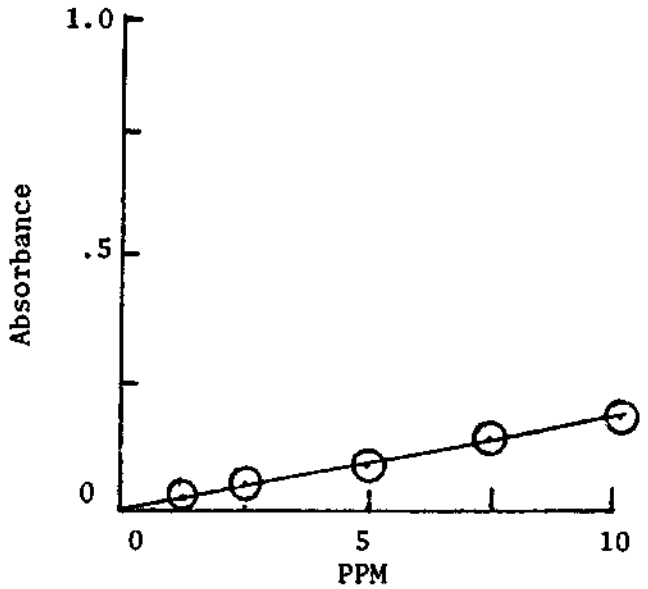
Disperse Red 60



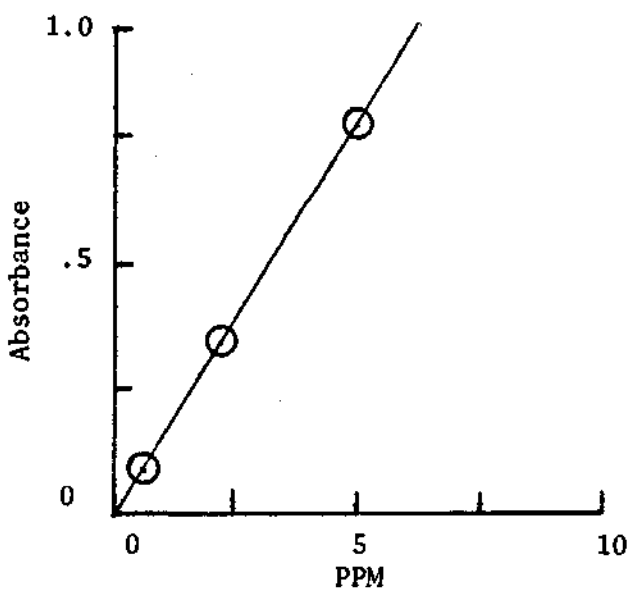
Disperse Yellow 3



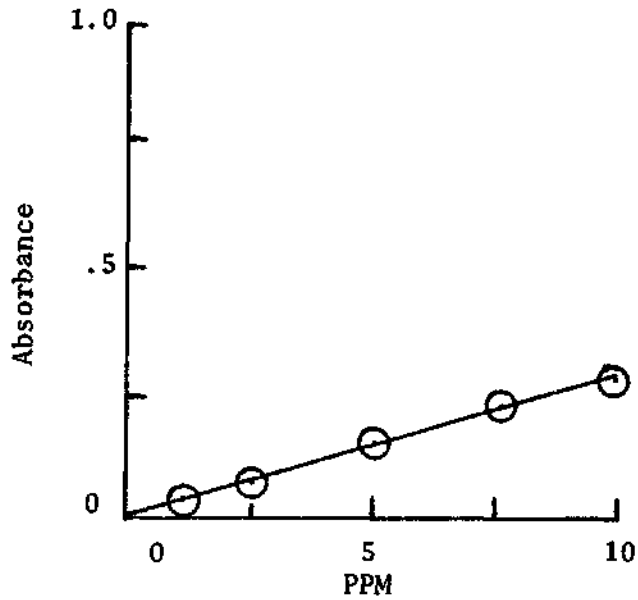
Disperse Blue 120



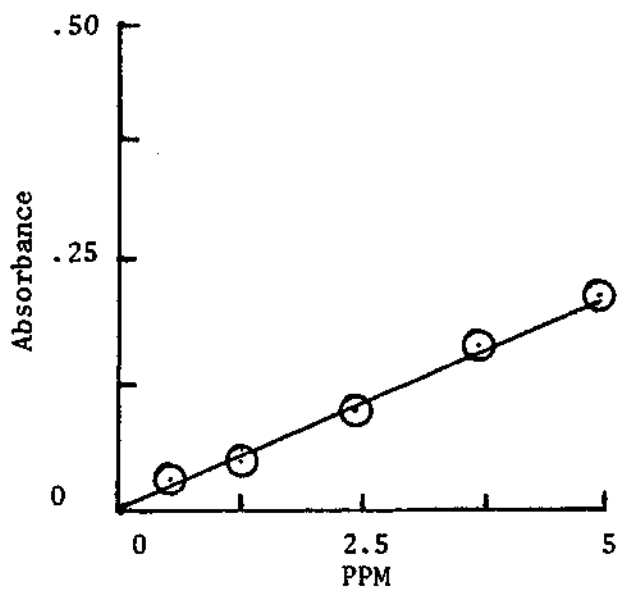
Disperse Blue 7



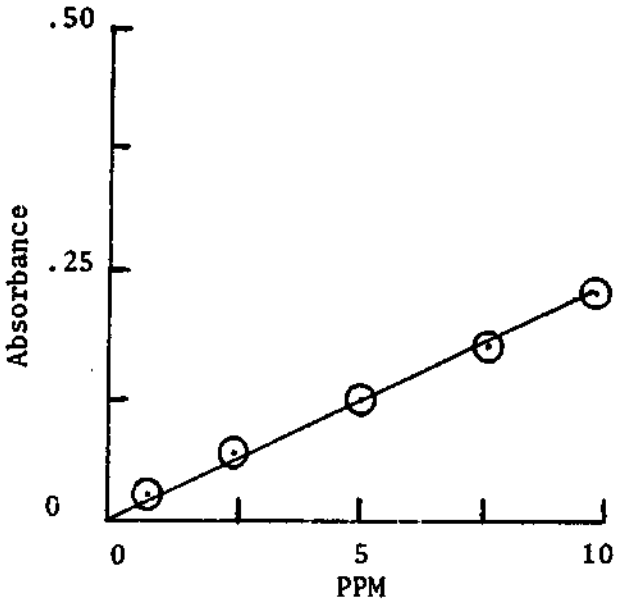
Disperse Yellow 54



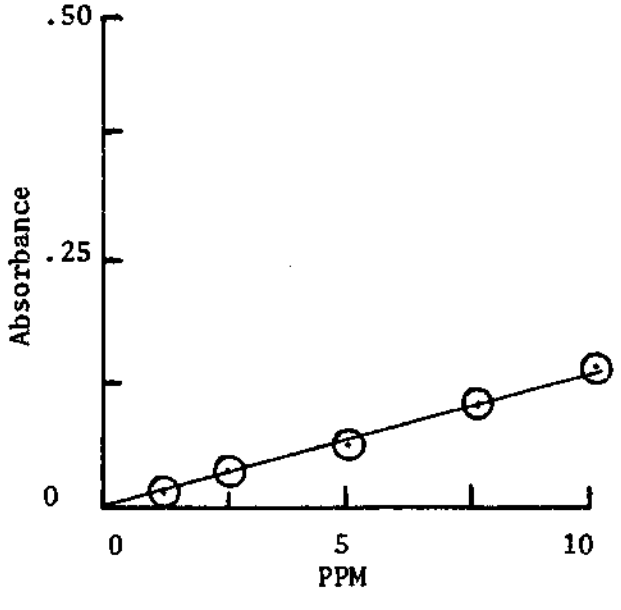
Disperse Red 55



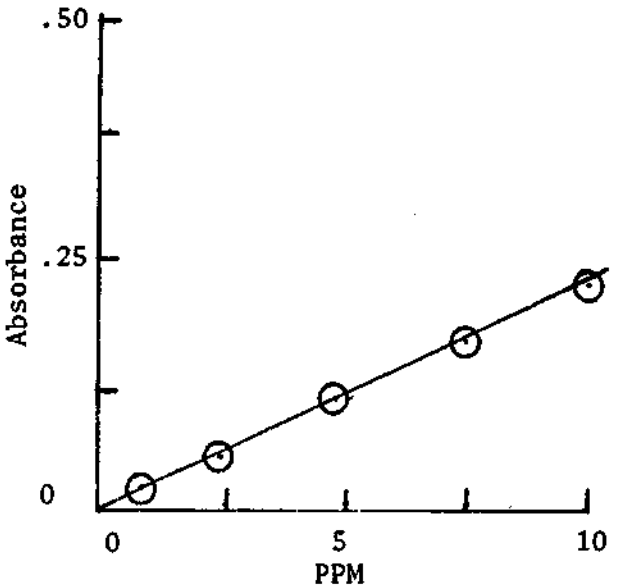
Acid Red 151



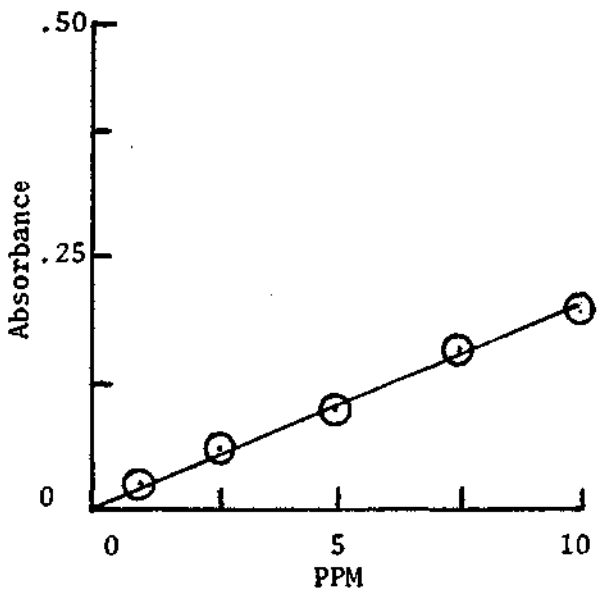
Acid Yellow 151



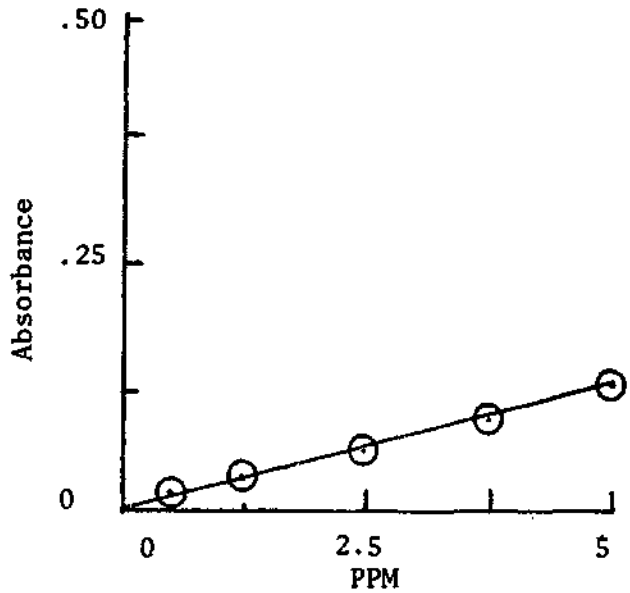
Acid Blue 40



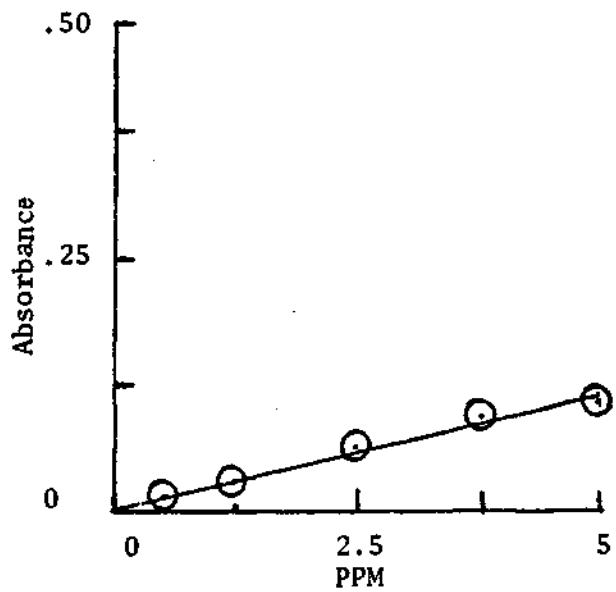
Acid Blue 25



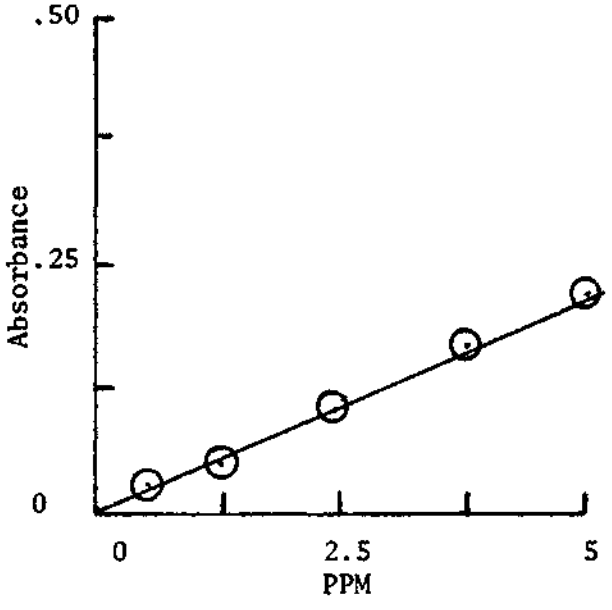
Acid Yellow 135



Acid Red 337



Acid Yellow 19



Acid Orange 128

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